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From slash, burn and poison to deployment of immune armies to treat cancers

A turning point has been reached in the 100-year history of cancer drugs: therapeutic strategies that aim to set free or augment the patients’ own immune system to fight off tumors are showing great promise.

Nobel Spotlight Part II:
Professor Phillip Sharp, MIT Department of Biology

The second installment of a MURJ interview with Professor Phillip Sharp, who discovered RNA splicing as an assistant Professor at MIT 36 years ago—for which he won the 1993 Nobel Prize in Medicine or Physiology—and has pushed forward biology at an extraordinary clip ever since.
**Biomechanics Effects of Running on Load Distribution to the Plantar Surface**

Dacie Manion, Peter So

Lower extremity injuries may be related to impact loads, which are affected by landing style, running shoe choice, terrain, and speed. In this study, the subject ran trials with varying shoe, surface, and speed conditions using a forefoot and then a heel strike landing style. In-shoe measurements of load imparted to the ball, arch, and heel were made using force sensing resistors.

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**Cross-Linking of a Positive-Tone, Polynorbornene Dielectric**

Helen Wendi Li, Zachary Dylan Pritchard, Paul Kohl, PhD

Dielectric films are often used in microelectromechanical systems (MEMS) and microelectronics packaging, where good mechanical and electrical properties are desired to improve device processability and reliability. The good mechanical properties are achieved by addition of an epoxy cross-linker, which forms covalent bonds between polymer chains (cross-links) at elevated temperatures. In this study, cross-linking in a positive tone, aqueous-developable, polynorbornene (PNB) dielectric was investigated.

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**Characterizing the Effect of Inactivating SIRT1 in Mouse Embryonic Stem Cells**

Amy K. Taylor, Eric O. Williams, Leonard P. Guarente

Embryonic stem (ES) cells have come to be recognized for their potential role in regenerative medicine. Since the discovery of induced pluripotent stem cells (iPSCs) in 2006, there has been an ongoing effort to improve the process of reprogramming differentiated cells. Improved understanding of pluripotency pathways will enable researchers to overcome the current barriers to efficient and safe reprogramming.

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**Characterizing MDSC Migration After Functionalization with Targeted Microparticles**

Rumya Raghavan, Kavya Rakhra, Darrell Irvine

Cancers are responsible for nearly 7.6 million deaths per year and unfortunately most leading chemotherapeutic treatments are highly toxic to healthy cells. In an attempt to combat this issue, controlled drug delivery systems are used in which drugs are transported to the site of the tumor, thereby limiting toxicity and dosage concentration (Srivasta et al. 2012). We propose to use myeloid derived suppressor cells (MDSCs) attached to polymer backpacks as a drug delivery vehicle to achieve targeted drug delivery to the tumor site.

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**The Characterization of Thin Film SnS for Solar Cell Applications**

Max Powers, Katherine Hartman, Tonio Buonassissi

Solar cell research is very important in the ongoing process to move away from fossil fuels as a primary global energy source. Currently a great quantity of solar cell research works to optimize Si (silicon) solar cells. Si solar cells are solar cells with their primary electron generation components made of Silicon. Factors that affect how efficient these solar cells work include: thickness, different kinds of anti-reflective coating, and doping to make it more efficient. The relative abundance of Si makes it an attractive choice for solar cells.

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**In this special volume, the MIT Undergraduate Journal (MURJ) features a research report co-authored by students Helen Li and Zachary Pritchard, two undergraduates from the Georgia Institute of Technology. See page 30 for a description and page 38 for the report!**
May 2014

Dear MIT Community,

At MIT, pushing the boundaries of human understanding is central to our work, and the world looks to us for pioneering research results. But MIT is equally dedicated to educating students, and for that reason, we also value research as a process. When students tackle the practical challenges of exploration, experimentation and discovery, they engage in a powerful form of “learning by doing” — and they experience a distinctive part of what it means to be educated at MIT.

Almost everywhere, research is the essence of graduate education. But since the 1969 launch of MIT’s Undergraduate Research Opportunities Program (UROP), research has come to be a pervasive and popular part of the MIT undergraduate experience, too. Today, more than 85 percent of our graduating seniors have worked with MIT faculty on frontline research. For those who go on to academic careers, such early research experiences have obvious benefits. But we believe that experiencing research from the inside can be transformative for all of our students, no matter how their careers unfold.

With undergraduates as junior colleagues in our laboratories, MIT faculty, post-docs and graduate students can naturally transmit the values, ethics and intellectual standards required for first-rate research. And our undergraduates can explore and reflect on the whole range of experience involved in advancing the scientific frontier: the challenges and pleasures of intellectual collaboration. The brilliant creative leaps and the dogged self-discipline. And, if they are lucky, the thrill of finding something new. Such skills and experiences can be hard to acquire in a classroom — but they constitute a very important aspect of the education available at MIT.

As you will see in the pages of MURJ, this intense educational process often leads our undergraduates to produce impressive research results. I am grateful that each issue of MURJ allows them to share their work with our community and the world.

Sincerely,

L. Rafael Reif
President of MIT
May 2014

Dear MIT community,

We are excited to present the 27th issue of the MIT Undergraduate Research Journal, a biannual student-run publication that features groundbreaking undergraduate research from across campus. As always, the research conducted by MIT undergraduates continues to push the boundaries of science, and the articles and summaries presented herein are evidence of this creative spirit. In this issue, we learn about two possible roles of the gene SIRT1, both in circadian rhythms and induced pluripotency, biomechanical insights to decrease running injuries, and a novel solar cell design. It is also our pleasure to feature an original research article from The Tower, the undergraduate research journal at the Georgia Institute of Technology, with whom we have been collaborating over the past year.

In addition to our research articles, we also present insightful features articles that explore exciting and emerging scientific topics. In this issue our features detail the quickly advancing field of cancer immunotherapy, where discoveries over the past decades are continuing to have a substantial impact on the lives of cancer patients. We also continue our Nobel Spotlight from our past issue, which explores Phil Sharp’s many accomplishments through an interview with our own staff.

During CPW 2014, MURJ hosted MIT’s first interdisciplinary undergraduate poster session in collaboration with the MIT chapter of Sigma Xi. The event was a phenomenal success, and we are pleased to include an article reviewing the event. This piece, along with others presented within, is available online at murj.mit.edu.
where we highlight the most recent additions to MIT’s history of discovery and innovation.

This journal is a collaborative effort by an extraordinary team of dedicated students, and we would like to thank all our editorial board and contributors for their time and effort this semester. It has been a pleasure to work alongside our hard-working staff, without whom this journal would not be possible. We would also like to thank all of the student researchers who graciously shared their research.

If you would like to contribute to future issues of the MIT Undergraduate Research Journal, we invite you to join our team of authors and editors or submit your research for our Fall 2014 issue. Please contact murj-officers@mit.edu if you have any questions or comments.

Best,

Elliot Akama-Garren  
(Co-Editor-in-Chief)

Ebaa Al-Obeidi  
(Co-Editor-in-Chief)

Sarine Shahmirian  
(Co-Editor-in-Chief)
The research group, led by Alexander Khitun, created a prototype of a 2-bit magnonic holographic device with a pair of magnets aligned in different positions on the magnetic waveguides. These magnets alter the spin waves going through the waveguides, and the resulting wave interference can create a clear image.

“The results open a new field of research,” said Khitun, “which may have tremendous impact on the development of new logic and memory devices.”

—A. Liu
Source: http://www.sciencedaily.com/releases/2014/02/140219142604.htm

NEUROSCIENCE

A Sleep Switch

The statement lost sleep may never be recovered could possibly be true. Researchers at Oxford University’s Centre for Neural Circuits and Behaviour have discovered that mutant fruit flies couldn’t catch up on their lost sleep after they were awake through the night. Researchers’ goal has been to understand the sleep switch in our brains to identify targets for drugs to improve treatments for sleep disorders.

Two mechanisms the body uses to regulate sleep are the 24-hour body cycle of day and night and the homeostat, a device in the brain that keeps track of waking hours and triggers sleep to reset. The sleep switch that researchers have identified in flies is a bundle of sleep-promoting neurons that fire when the body is tired and needs sleep, according to Professor Gero Miesenböck, whose lab performed this research. This neuron bundle is a critical part of the sleep mechanism as observed in experiments with the mutant flies. In mutant flies, sleep-inducing neurons were constitutively inactive, causing insomnia, and electrical activity of the homeostat nerve cells was broken. Though the data on the sleep switch was based on experiments with flies, Dr. Jeffrey Donlea, one of the lead authors of the study explained, “There is a similar group of neurons in a region of the human brain.” These neurons are targets of anesthetics, so it’s likely these neurons have a similar mechanism to the ones discovered in the flies.

Now that the critical component has been pinpointed, researchers are working to find internal signals that the sleep switch responds to. Other unanswered questions that research will hopefully one day find answers for are why we need sleep at all and how the brain tracks waking hours. Researchers are optimistic that we are taking step forwards to understanding the mystery of sleep.

—L. Subbaraj
Source: http://www.sciencedaily.com/releases/2014/02/140219142730.htm
**Jazz Improvisation Linked to Areas in the Brain that Process Language**

A novel study conducted by researchers at Johns Hopkins University has demonstrated that improvisational music conversation taps into the same areas of the brain that process syntax in spoken language.

Researchers used functional magnetic resonance imaging (fMRI) technology to track the activation in different parts of the brain of jazz musicians. In the study, jazz pianists played an act of “trading fours,” a spontaneous back and forth improvisational act, while being observed under fMRI.

“When two jazz musicians seem lost in thought while trading fours, they aren’t simply waiting for their turn to play,” said Limb, the senior author of the study. “Instead, they are using syntactical areas of their brain to process what they are hearing so they can respond by playing a new series of notes that hasn’t previously been composed or practiced.”

The results revealed that the improvisation between the musicians activated the inferior frontal gyrus and posterior superior temporal gyrus of the brain. These are regions of the brain that are used in syntactical processing, which is the ability to interpret the structure of language. However, the improvisation did not activate areas of the brain involved in the processing of semantics, the ability to find the meaning of spoken language.

The study offers new insight on how we process auditory information that we take in from the world around us. “Until now, studies of how the brain processes auditory communication has been done only in the context of spoken language,” said Limb. “Looking at jazz lets us investigate the neurological basis of interactive, musical communication as it occurs outside of spoken knowledge.”

—A. Liu

**Dogs Are Sensitive to Human Emotion**

The human brain has a “voice area” that allows us to associate a person with their voice, picture that person when hearing them, and read emotions in the voice. Research has shown that the dog has a voice area as well, which explains why they are so attuned to their owners’ emotions.

Attila Andics, a neuroscientist at Hungarian Academy of Sciences, and his team trained dogs to lie motionless in an MRI scanner while wearing head-phones that played different dog and human sounds, such as whines, cries, playful noises, and laughs.

The MRI scanner captured images of dogs’ brain activity, which were then compared to humans’ brain activity when people were scanned under the same conditions.

Andics and his team discovered that dogs also have a voice area in their brain and that humans and dogs have similar brain mechanisms in processing the social meaning of sound.

This new insight explains why dogs seem to respond to how we say something and understand the emotions behind our words even though they may not necessarily understand exactly what we are saying.

—R. Park

**Studies show that jazz improvisation activates in the brain areas that process syntax.**

Credit: http://upload.wikimedia.org/wikipedia/commons/7/7c/Freddie_Hubbard_1976.jpg

**Brain scans of dogs show similar vocal processing areas to those of humans**

Credit: http://news.sciencemag.org/sites/default/files/styles/thumb_article_1/public/sn-dogs.jpg
MEDICINE

Promising Future for Cell Therapy

Cell-based therapy, or targeted immunotherapy, is a promising new way to treat cancer that involves using the patient’s own immune system to attack cancerous cells. Our immune system does not recognize cancer cells as foreign, so it does not eradicate cancer like it does with common viruses. The goal of many researchers has been to explore methods to engineer T-cells to recognize and kill cancer cells.

Researchers at Memorial Sloan Kettering are the first to target CD19, a surface protein of B-cells that T-cells have been engineered to recognize to treat B-cell cancers. In their clinical study, they examined patients with relapsed adult B-cell acute lymphoblastic leukemia (B-ALL). B-ALL is currently difficult to treat because most patients relapse after various treatments, like chemotherapy. The only hope for long-term survival is a successful bone marrow transplant. In the study, 16 patients were given an infusion of their own genetically modified T-cells “programmed” to recognize cancerous B-cells with protein CD19. The complete response rate, at 88 percent, for patients with this treatment far exceeded the complete response rate for those with chemotherapy alone. Researchers later reported that all five patients who were treated with cell therapy achieved complete remissions. One patient had a successful bone marrow transplant after receiving cell therapy two years earlier and is now cancer-free.

Dr. Michel Sadelain, Director of the Center for Cell Engineering at Memorial Sloan Kettering said, “Initial findings have held up in a larger cohort of patients, and we are already looking at new clinical studies to advance this novel therapeutic approach in fighting cancer.” With this news, additional studies examining if B-ALL patients should receive targeted immunotherapy at the beginning of their treatment and if similar cell therapy can be applied to other cancers are being planned in the hopes that this exciting new method can be used for many patients in the future.

—L. Subbaraj

Source: http://www.sciencedaily.com/releases/2014/02/140219142556.htm

CRISPR/Cas9: A Versatile Tool for Genome Editing

In 1987, a team of Japanese bacteriologists discovered in E. coli several palindromic repeats of DNA nucleotides separated by different “spacer” sequences. At the time, the researchers had no idea about the biological significance of these repeat sequences, which were later termed “clustered regularly interspaced short palindromic repeats” (CRISPR), and merely reported them in a “by the way” paragraph.

In 2007, scientists determined that the purpose of CRISPR is to function as bacteria’s adaptive immune system against viral insults. The spacer DNA sequences can be converted to RNA strands that match certain genetic parts of viruses which have previously infected the bacterium or its ancestor. The guide RNA (gRNA) associates with an enzyme called Cas9 and delivers it to the matching gene, where Cas9 cuts the DNA on both strands, effectively knocking out the gene.

By 2013, scientists around the globe have witnessed the power of the CRISPR/Cas9 system as a customizable genome-editing tool. Researchers in China, for example, have made CRISPR knockout monkeys with two target genes disrupted at once.

GENETIC ENGINEERING

A customizable piece of RNA (green) delivers the Cas9 enzyme (purple) to the target DNA (blue), where Cas9 creates a double strand break that effectively knocks out a gene.

Credit: http://pnabio.com/products/RGEN
But the capability of CRISPR/Cas9 goes beyond just knocking out genes. In fact, one can also change or repair genes with this tool.

Last year, Dutch researchers successfully utilized CRISPR/Cas9 to correct the most common disease-causing mutation in Cystic Fibrosis, ΔF508 on the chloride channel CFTR, in patients’ stem cells—by supplying not only a gRNA but also a donor DNA plasmid which encodes the wild-type CFTR.

In the coming decade, CRISPR/Cas9 is bound to extensively influence not only how basic scientists perform biological research but also how industrial and medical professionals pursue therapeutic strategies.

—J. Chen

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Molecular identity of mitochondrial Ca^{2+} transport channel unveiled

Mitochondria, the energy power plants of the cell, are responsible for producing most of the cell’s supply of ATP. These energy factories have been shown to be capable of uptaking a large amount of calcium ions (Ca^{2+})—so much calcium that, at maximum loading, a quarter of the dry weight of a mitochondrion is calcium phosphate. This calcium uptake stimulates ATP production, and defects in mitochondria’s Ca^{2+} transport machinery can have profound clinical implications. The molecular identity of the Ca^{2+} channel on mitochondrial membrane, however, has been elusive until very recently, partly because of the complexity of mitochondria, which contain 1200+ proteins.

In 2010, Dr. Vamsi Mootha and colleagues at MGH took advantage of the fact that S. cerevisiae (yeast) mitochondria, while sharing lots of homologous genes with human mitochondria, cannot uptake calcium—which allowed the researchers to narrow potential candidates for the Ca^{2+} channel down to the 58 non-homologous genes. They hence conducted a focused RNAi screen and found that silencing M1CU1 abolished calcium entry into mitochondria. However, the MICU1 protein lacks transmembrane domains and therefore is only a regulator rather than the Ca^{2+} channel itself.

In a follow-up study in 2013, the researchers performed elegant computational studies to check for other genes whose mRNA co-express with MICU1 in the whole genome, whose proteins co-express with MICU1 in the mitochondria, and whose homologues co-exist with those of MICU1 across 500 evolutionarily diverse organisms.

All three assays converged on a clear winner gene named MCU, the “Michael Phelps of genomic assays,” as Prof. Mootha put it. Combining this data with knockdown, biochemical and structural studies, the researchers have unequivocally established the molecular identity of the mitochondrial Ca^{2+} uniporter.

—J. Chen

Sources:
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http://www.nature.com/nature/journal/v476/n7360/full/nature10234.html
Paralysis of the muscles can be caused by many things, including but not limited to injury, stroke, or other forms of disease. In order to treat this, scientists are inventing brain-machine interfaces that transform electrical signals in the brain to movement in a device like a robotic limb or even the patient’s own paralyzed limb.

In paralyzed patients, the neurons in the brain’s motor cortical areas are still activated when a movement is imagined or planned. However, the communication link between the brain and the spinal cord (and therefore the muscles) is broken.

Maryam Shanechi, a professor of EE and CS at Cornell, and Ziv Williams, a professor of neurosurgery at Harvard Medical School, are currently conducting research on brain-machine interfaces that allow patients to overcome this disconnect between the brain signals and the limbs due to paralysis.

The brain-machine interface is based on real-time decoding of neural signals into movement. One animal would act as the controller and determine what it wants to do and the decoder would stimulate the spinal cord of a sedated animal to produce desired movement.

Shanechi and Williams et al. are by no means curing paralysis, but they are using the concept of the brain-machine interface to allow patients to control their own paralyzed limb by implanting sensors which act as decoders that record and interpret neural activity and produce intended movement in the paralyzed limbs.

—R. Park

Source: http://www.sciencedaily.com/releases/2014/02/140219124730.htm

A diagram of a prosthetic arm controlled by neural signals. Shanechi and Williams are using this concept to allow patients to control their own paralyzed limb.

Credit: Credit: http://images.gizmag.com/gallery_lrg/thought_controlled_permanent_prosthetic_arm-2.jpg

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Emma Whitehead, a 5-year-old girl living in Pennsylvania, had fallen terribly sick with acute lymphoblastic leukemia (ALL). The doctors had tried two rounds of chemotherapy but her tumors still came back, each time more aggressively than before, bringing Emma close to death. It was then that her parents signed the consent form for an experimental approach called “chimeric antigen receptor” (CAR)-modified T cell therapy. Despite some initial struggles, Emma beat her cancer using nothing but her own T cells.¹

The experimental approach involved removing Emma’s T cells, engineering them using an altered form of HIV capable of encoding any genes of interest, and re-infusing the modified T cells back into her body. The T cells were reprogrammed to express a new T-cell receptor specific for the B cell antigen CD19, as her leukemia was caused by uncontrolled proliferation and invasion of B cells. In addition, the newly installed receptor on the T cells was equipped with a signaling domain called CD3-zeta and a co-stimulatory domain called 4-1BB, both of which delivered growth signals to help the engineered T cells replicate once in the body.² Indeed, this specific clone of T cells massively expanded to 1,000 fold of their initial count, while their targets, the cancer-causing B cells, dropped to essentially zero in three short weeks. Emma has been cancer-free by any measure since receiving this treatment in 2012.

The CAR therapy falls under the broad category of many paradigm-shifting treatment strategies termed cancer immunotherapy, which aims to activate or unleash the patients’ own immune system to fight off tumors. Another successful example of cancer immunotherapy is called “checkpoint blockade” and includes such therapeutic antibodies as anti-CTLA4 and anti-PD-1.³

Ironically, the scientific rationale behind these checkpoint antibodies was not focused on cancer cells per se. “Forget about the tumors; treat the immune system instead,” said Dr. James Allison, who discovered the inhibitory function of CTLA-4, a surface molecule on T cells.⁴ In order for T cells to properly function, two signals are required: an antigen (the “danger signal”) and a co-stimulatory signal. CD28 is one such co-stimulatory receptor on T cells.⁴ The engagement of CD28 with a ligand called B7 on the cells that present the antigen allows
T cells to activate and survive. When CTLA-4, a CD28 look-alike, was initially identified, it was assumed that CTLA-4 was simply another co-stimulatory receptor which also helps deliver the survival and activation signals.\textsuperscript{5}

The truth could not have been more different, nor the significance more far-reaching. Allison showed that CTLA-4 was actually an inhibitory molecule which competes with CD28 for B7, and CTLA-4 often wins out due to its stronger binding affinity.\textsuperscript{6} A T cell thus evaluates both the inhibitory signal delivered by CTLA-4 and the activating signal by CD28 and integrates the two, not unlike how a neuron integrates both GABA-ergic (inhibitory) and glutamatergic (excitatory) signals in deciding whether to fire an action potential. The logic then follows that by inhibiting the inhibitory receptor CTLA-4 with a monoclonal antibody, the T cells could be set free to perform their cytotoxic function against tumor cells.

Yervoy, an anti-CTLA-4 antibody developed by Bristol-Myers Squibb, was approved in 2011 and quickly became a frontline therapy for metastatic melanoma.\textsuperscript{7} Five years ago, a diagnosis of advanced melanoma was in essence a death sentence. But today, many patients can benefit from this therapeutic antibody, the first ever drug to show prolonged survival in patients with the advanced cancer.

It is hardly surprising that PD-1, yet another inhibitory receptor on T cells, also became an attractive target for therapeutic intervention. In Phase II clinical trials, an anti-PD-1 antibody was shown to shrink tumor mass by at least 50% in melanoma, kidney cancer and lung cancer.\textsuperscript{8} It was unheard of in the history of human cancer therapies for a non-chemotherapeutic drug to exhibit efficacy against three diverse oncology indications. Moreover, combination of anti-PD-1 and anti-CTLA showed synergy: the percentage of patient survival doubled from the low 20’s with single therapy to the mid-40’s with combination of the two.\textsuperscript{9}

The stellar performance of these checkpoint blockade therapies has turned skeptic immunologists into enthusiastic believers. Dr. Hidde Ploegh, professor of biology at MIT and recipient of the 2012 NIH Director’s Pioneer Award, told a room-full of scientists in a 2013 international conference held at the Cold Spring Harbor Laboratory: “As a non-religious person, I don’t attend church and I don’t believe. But if given the choice between prayer and immunotherapy five years ago, I’d choose prayer any time.” After seeing the recent data of the remarkable patient responses, however, Prof. Ploegh now regularly relates the CTLA-4 story to fellow scientists and students alike.

In the context of checkpoint blockade, immunotherapy has two important advantages. First, since the therapy augments the patient’s own immune cells, the nature of the tumors becomes essentially irrelevant. With inherent mechanisms to generate an incredibly diverse repertoire, T cells can in principle fight just about any tumor, as long as they

"Five years ago, a diagnosis of advanced melanoma was in essence a death sentence. But today, many patients can benefit from this therapeutic antibody [...]"
are given the green light to do so. “It’s like giving the T cells steroids and allowing them to do what they otherwise cannot do,” Ploegh drew the analogy. The universal applicability of immunotherapies like checkpoint blockade surpasses the limited utility of targeted therapies, where each drug is only effective for a fraction of a specific type of cancer. As an example, Herceptin (a monoclonal antibody for breast cancer treatment) is only helpful for the 20-25% of breast cancer patients whose tumor cells overexpress the growth factor receptor HER2.

Secondly, the effect of immunotherapy can be “long-lasting, especially if memory T and B cells can be generated,” said Dr. Jianzhu Chen, Cottrell Professor of Immunology at MIT and a principal investigator at the Koch Institute. Immunological memory is the key reason why vaccines against infectious diseases like smallpox and measles work. After an initial exposure to antigens, subsets of T and B cells differentiate into memory cells, which can quickly recall the first encounter and mount an even stronger attack in case the same antigens reappear. It is no different for tumor antigens. When T cells become equipped with the ability to recognize and attack antigen-bearing tumor cells, some of these T cells remember this event and therefore can respond rapidly if the tumor comes back.

Dr. Allison often proudly shares the story of a patient named Sharon, who suffered from a “grapefruit-sized tumor” in her lung from melanoma and received a “single dose of anti-CTLA-4” in 2001 in an early clinical trial. She emerged cancer-free and has been living a healthy life ever since without any continuing therapy. This is effectively a complete “cure,” although the word is almost a taboo among oncology doctors.

In the scheme of the two-signal model for T cell activation, checkpoint blockade therapies focus on the second signal (co-stimulation) while assuming the first signal derived from antigen presentation is intact and functional. However, antigen processing and presentation is a complex mechanism that is often hindered by tumors. For example, many cancer cells have been found to down-regulate their surface expression of Class I MHC, the very molecule that presents tumor antigens to T cells. This interference of MHC expression is an important mechanism through which tumors escape immune surveillance.

A German biotech company named Micromet came up with an elegant solution to this problem: an antibody with two specificities. The antibody, which was named “bi-specific T cell engager” (or “BiTE” for short), targets a tumor antigen on one end and binds T cells on the other end. Therefore, the BiTE molecule serves as an adaptor that glues T cells directly onto the cancer cells, concentrating the full force of the most potent killers in the body on the tumors.

"BiTE molecule serves as an adapter that glues T cells directly onto the cancer cells, concentrating the full force of the most potent killers in the body on the tumors."

and cancer researchers. What is indisputable though is that the re-engineered or unleashed T cells are essentially “living drugs,” said Dr. Michael Sadelain, director of the Center for Cell Engineering at Memorial Sloan-Kettering Cancer Center in New York. Such living drugs differ from pills, which get “eliminated from your body and you have to take them again.”
recognize specific tumor antigens are no longer required—any killer T cells can be recruited by BiTEs to destroy tumors.\(^\text{13}\)

Since the BiTE antibody is simply a guide for T cells and not an actual “drug” that kills tumors, only a very small dose is required for efficacy. While typical therapeutic antibodies are administered to patients in the milligram range (2-4 mg/kg for Herceptin), micrograms of BiTEs are sufficient to achieve remarkable responses. In a 2012 phase II clinical trial which tested the efficacy of Blinatumomab—a BiTE developed by Amgen that brings T cells to CD19-expressing B cells—72% of acute lymphoblastic leukemia patients recorded a complete response, meaning all their leukemic cells disappeared.\(^\text{14}\)

The dose of the BiTE used in this study was hundreds of times lower than the required dosage of any current therapeutic antibodies on the market.

The deployment of T cells to kill tumor cells that express an antigen of choice is a common theme to both the CAR-modified T cell therapy and BiTEs. However, there are major differences from the operational viewpoint. The CAR therapy requires removing T cells from each patient, modifying them and re-infusing them back to the patient—an overall labor-intensive, time-consuming and expensive procedure. Many steps could go wrong along the way. In fact, the Penn team suspected “a flawed batch of T cells” as a possible reason when explaining why the CAR therapy didn’t work at all in some patients. Besides, since T cells cannot be cross-transfused among different patients, this complicated procedure must be performed again for a different patient, making it “personalized medicine.” On the contrary, protein therapies like BiTEs can be mass-manufactured, universally applied and more easily administered.

In addition to T cells, there are other immune cell types which have been utilized in various immunotherapeutic approaches. Dendritic cells (DCs) are professional antigen-presenting cells that serve as the sentinels of the immune system, constantly sampling their environment for signals of danger and duly reporting them to the adaptive immune cells. Due to their role in antigen presentation, DCs have been attractive targets for therapeutic intervention. Provenge, a DC-based therapy for advanced prostate cancer, was approved by the FDA in 2010.\(^\text{15}\)

The approach taken by Provenge involves isolating a patient’s dendritic cells, loading them with a prostate tumor antigen called PAP, and re-infusing the cells back into the patients. The engineered DCs can then elicit a strong adaptive immune response against the PAP-expressing prostate cancer cells. While effective and life-extending, this therapy (like CAR-modified T cells) entails sophisticated procedures that need to be conducted for each patient.

To enhance antigen presentation by DCs without removing them from the body, several research teams have pursued a different strategy that involves the targeting of Dec-205, an antigen uptake receptor on the surface of DCs.\(^\text{16}\) By fusing a tumor antigen to an antibody that binds Dec-205, the antigen can be accurately delivered to DCs and promptly accepted through the normal function of Dec-205. This approach has been shown to induce strong anti-tumor immunity—both humoral (production of antibodies) and cellular (production of functional T cells)—in mice as well as non-human primates.\(^\text{17}\)

Despite all these successes in utilizing the immune system to combat tumors, not all immune cells are helpful for this goal. In fact, several immune cell types evidently play a counterproductive role in anti-tumor immunity. Regulatory T cells (Tregs for short) and macrophages, for example, are among the troubling cell types that bring headaches to cancer immunologists.\(^\text{18}\)
Tregs normally function to dampen T cell response, as massive proliferation and excessive action of T cells are energetically expensive and potentially dangerous. In the case of anti-tumor immunity, however, Tregs often come into the picture too early, shutting down T cells that have not finished their task of tumor destruction.

Macrophages, on the other hand, normally play an important role in inflammation through secretion of pro-inflammatory signals and recruitment of additional immune cells. However, infiltration of tumor sites by macrophages has been negatively correlated with long-term prognosis. Whereas patient biopsies in which a high number of killer T cells are observed often translate into long-term survival, the opposite is true for macrophages: tumors that are highly enriched by macrophages are far more likely to kill the patient. This immune typing of tumor sites has proven to be more useful than traditional staging of cancers in predicting long-term outcome.

Patients who have a Stage II tumor infiltrated by many killer T cells and few macrophages will likely fare better than those who have a Stage I tumor that is filled with macrophages but few T cells.

The lesson from these studies is two-fold: that it is crucial to continue basic research to further understand the mechanisms through which Tregs and myeloid cells like macrophages act to impair anti-tumor immunity; and that it is plausible to target inflammation as an additional method to treat cancers.

As of today, the most common approaches to control cancers are surgery, radiation and chemotherapy. While surgery is useful for removing the bulk mass of tumors, any residual disease will likely lead to relapse in a more aggressive form. While fast-acting and powerful, radiation and chemotherapy are often very toxic and induce unbearable side effects. To be sure, the myriad cancer immunotherapies have not replaced these traditional approaches of slash, burn, and poison, but they do provide doctors and patients with additional options that are often more precise, more manageable and longer-lasting.

The four cardinal features of the adaptive immune system are diversity, specificity, memory and tolerance; each cancer immunotherapeutic approach has capitalized on at least one of these important features. Diversity is featured in checkpoint blockade therapies, which enable T cells to respond to essentially any immunogenic tumor. Specificity is exhibited by CAR-modified T cells and BiTEs, which promote the killing of specific antigen-expressing tumor cells by engineered or recruited T cells. Memory is attested by stories like that of Sharon, who received a single dose of anti-CTLA-4 and remained disease-free for more than a decade. However, tolerance, or the ability of the immune system to tolerate harmless self-antigens while acting on dangerous tumor antigens, is the one aspect in which cancer immunotherapy has not fared so well.

Tolerance is typically useful for distinguishing host molecules from foreign ones (such as those of infectious pathogens). Cancer cells, however, are still strictly speaking “self”—they are simply host cells that have gone wild in terms of growth and division. Therefore, tumor-associated antigens are often found on their healthy counterparts too. It was no surprise that Emma’s CAR-powered T cells also attacked her normal B cells and induced severe fever as a result of massive inflammatory cytokine release. Luckily, timely treatment with a cytokine antagonist alleviated the symptoms and kept Emma alive while her immune system gradually completed the battle against the leukemia.

Similarly, the anti-CTLA-4 antibody used to treat melanoma has been found to affect melanocytes in general. Since melanocytes produce melanin, a pigment found in the skin and hair, depigmentation often occurs in patients treated with this drug. The practice of immunotherapy, therefore, requires quick identification of and prompt response to any adverse autoimmune responses.

The success of cancer immunotherapies in recent years has turned heads and opened eyes. They are innovative and effective ways to treat cancers, but they are not perfect. Like most other drugs, there is a trade-off between efficacy and side-effects. However, most people would agree that therapies like anti-CTLA4 and anti-PD-1 have passed the risk-benefit analysis with flying colors. When one is facing painful death from metastatic tumors, the risk of hair depigmentation in exchange for a healthy life back will most probably seem like a great deal.
Nobel Spotlight Part II: Professor Phillip Sharp
MIT Department of Biology

This issue’s Nobel Spotlight features the second part of last issue's interview with Professor Phillip Sharp, 1993 Nobel Prize winner in Physiology or Medicine.

By Reuben Saunders, Riley Drake, & Semon Rezchikov

In 1974, Salvador Luria hired several talented young biologists to work at the MIT Center for Cancer Research, which he had just founded. Among them was Phillip Allen Sharp, a chemist and biologist with a strong interest in tumor-causing viruses. 39 years later, Sharp is an Institute Professor at MIT and a member of the Koch Institute for Integrative Cancer Research. As an assistant Professor, he discovered RNA splicing, the excision of introns from nuclear RNA to form messenger RNA, or mRNA, and the pace of advancement in his lab has scarcely slowed since. For his work on splicing, he won the 1993 Nobel Prize in Physiology or Medicine. He also cofounded Biogen (now Biogen Idec, after a merger), which is today the fourth largest biotechnology company. Recently, MURJ spoke with Professor Sharp.

In Part I of this interview, which was in the previous issue of MURJ, we discussed Professor Sharp’s life in science. In Part II of this interview, we discuss the nature of innovation in the sciences.

MURJ: “Biology is a much bigger field with respect to money spent on it, number of people working in it, technologies developed to work in it, etc. What has it been like working through the rise of molecular biology?”

Sharp: Absolutely fascinating. I came to MIT in ’74. In ’76, the community had discovered how to synthesize genes. Goban Korana did that here—he got absolutely no credit for it, until he got all the credit for it. It was also discovered how to recombine genes and how to sequence genes, all in the mid 70s. At the same time, there were moratoriums on recombinant DNA and genetic engineering, Cambridge moreso than anywhere else in the country—they shut us down completely for a year or two. But it was clear that there was this new technology of very fundamental promise for discovery in the laboratory and also translational science.

Venture capitalists called me and asked me to do a consult on a new and possible investment. I flew to the West Coast, and in walked Herb Boyer, Swan, Riggs, etc. This was the first investment in Genentech. I looked at the science and I said “The science being proposed, I don’t know if you can make a buck on this but they are going to do this science. They made the investment, and made a hardy return on it. My wife keeps reminding me how naive I was not to ask for some stock.

We returned to the East Coast and the dialogue continued, and that let both Wally Gilbert at Harvard and me to founding Biogen. We were 6, 8 scientists all working together to found Biogen. We wanted to start a US headquarters, and Wally Gilbert and I walked around Cambridge, we wanted it to be in Cambridge, we went over to Binney Street, there was this old factory there, it was dilapidated,
but it was close to MIT, it was close to the T station at Kendall Square, it was available, and it was the right size, so we took it and renovated it and that became the headquarters of Biogen, and then over the next 35 years Biogen was grown into, I was on the board for 29 years and chair of the scientific advising board, I was involved as a consultant, I never left MIT as a faculty member. It’s now, it went up another 13% last week, but it should be probably worth $50 billion now, maybe more, with 7000 employees. It’s produced a Hepatitis B vaccine intellectual property, alpha-interferon, a major treatment for Hepatitis B and C, and we’re the first, biggest market in market in multiple sclerosis, avenex, and the second and third lines avenex. With the merger with Idec, it’s one of the major cancer therapies. It’s an incredibly successful company, and it’s fascinating watching all that happen.

Then you started seeing more investment by big pharma in molecular and cell biology, you realize biology is an emerging science with all sorts of new discoveries. Molecular biology didn’t start until ’53, and it took us 20 years to learn how to do recombinant DNA—we were interested in what genes do, so we made all this science to work with genes—the first synthetic biology was in the mid ’70s, and now we have the human genome sequence and can sequence all of life forms, and we will. Everything out there is DNA. I look at that plant, that plant is DNA. That’s what it is. It’s got a history, its all in its DNA, there’s 20,000 to 50,000 new genes, we can relate some of them to use, but its really a different setup, we just understood how those genes are manipulated by RNA to make that three dimensional plant, that’s 5 years old. That’s how new this biology is. We now sequence microbiomes, the biosphere, we’ve got an enormous collection of genes which have different properties, some of which we will use to solve major problems—food, energy, environment, and health. We’re at a “yes, biology has expanded enormously as new technology has happened and new discoveries have happened, but we’re only at a very early stage in seeing that happen. The best indication of that is that over the last 10 years or so the number of engineers at MIT who are working in working in biological materials has just skyrocketed, it’s a third of the faculty now—we’re seeing engineers recognize that there are all sorts of opportunities to take this science into new realms of translation and to practical problems, and that’s going to bring us a whole host of other developments and even greater community in life science. A very important story to think about in terms of what the ultimate impact of this is all going to be, we’re still very early in the science, and the last example I’ll give to that is that it wasn’t until 1998 that we realized RNA interference existed. That’s Andy Fire and Craig Mello, and Andy Fire was my graduate student and so obviously deep in my heart, and a good friend, but he did that after he left here, but that’s life—when I say that plant, how it becomes 3 dimensional, we didn’t understand that at all until about 5 years ago do the discovering of RNAi and small RNAs because that plant uses small RNAs in ways you just don’t even think about in mammalian tissue. It’s really fundamentally new, and it was clear to me and my colleagues here at MIT that because every cell in our body had the pathway related to RNA interference, the microRNA pathway, which David Bartell here made very important contributions to, we could treat genes in any cell with small RNAs, if we could get the RNAs into those cells. Almost all of your therapies are gene specific—you take statin for cholesterol and it’s one specific gene, and it inhibits that gene, that leads to lower cholesterol—that’s true of all other drugs, the more specific they are to one gene product the more valuable they are. So, can we take these RNAs and make them into genes for creating therapeutics, and that’s Amylum, which I started with my colleagues here in 2002, its 10 years old, and its doing very well. It’s not got a product on the market, but I think I’m going to change pharmaceutical chemistry.

MURJ: You were talking about how we are going to sequence all of the genomes. There’s been a lot of big biology in the past 15 years, with the Human Genome Project, and the Obama’s Brain initiative, but some of your most important results come out of very small, very targeted experiments by personally led investigative teams. How do you think these two approaches differ, and what should the balance be?
Sharp: I think we have to have both. As biology has become more powerful from a technology point of view, being able to automate things to do higher throughput, to collect more data electronically, you have to put together larger groups to do that. There’s been a lot of that done. The Broad here is sort of the master of that world. We do what we want to as groups of people, and when you go across the street to Biotech, the way that Biotech has to work, is that everybody, 50, 100, 150 people, I think that’s the optimal size, everybody knows everything going on and they work together as a team. That’s how they work, but that’s one of the reasons big pharma doesn’t do discovery well. They can’t focus on the individual in the laboratory. One of the things an academic does much better than big science is that the academic individual, and the lab, and the colleagues, and the setting around talking, so I think you have to have both structures. We’re struggling with getting the balance in a time in which there is a reduction in the amount of money being invested. These are not pleasant times.

We’ve got a federal budget deadlock with about equal evil on both sides, where one side wants to expand federal investment in a whole host of entitlement programs and labor programs, and one side wants more conservative federal budgets and more individual state rights, a discussion that’s been going on since the beginning of the country, but they are at absolute loggerheads and science is getting killed as these two elephants stomp around and butt each other. Our budgets are going down, not because they don’t like science and technology, they are actually quite supportive on both sides of the aisle, but they don’t want to give up this philosophical and economic fight, so it’s really damaging. If it continues very long, it’s going to be very damaging, not only because it’s going to reduce the amount of investment in innovation, but also because it’s going to turn a lot of students into other avenues, and they won’t come back, and because demographically the baby boomer retirements are looming, there are going to be lots of opportunities for you guys and gals in four or five years, ten years.

MURJ: There’s been a lot of talk about how these big science projects produce extremely large treasure chests of data, but then this data is somewhat decontextualized and is very hard to interpret. Do you have any thoughts as to how best do research on large teams?

Sharp: There are many ways of cutting this. Contextualized experiments should be supported individually. As an experiment becomes more contextualized, the answer is highly dependent upon the specifics of the experiment and it is not scalable in terms of being used broadly because you don’t know exactly how the experiment is done. I’ll give an example: imaging cells. You put cells in culture, they have a certain morphology, they change in that morphology, you can take pictures of them and everything else, but those pictures mean very little to others because it depends on the media, the serum, the time since the cell has been passed, all those sorts of things that are contextual. When you go to sequence the human genome, on the other hand, there are variations between us, but the major issue you are going to contribute is that this is a set of genes, genes that can be found anywhere on earth, by sequencing anybody, and that is 99% of the value of the operation, so it can be big science. Those are the two extremes. You can put different levels in the middle.
The other thing I would say is that, if you read a lot about new innovation, even innovation in large teams, the really critical ideas come from a few people. Sometimes a large team can be going this way, and they see something coming, and they don’t know how to overcome it, and they’ll take their best idea and put everybody on it and if they have good leaders, they’ll say “why don’t you explore this; why don’t you explore that,” and they’ll listen to what individuals are saying, and if it sounds as if that becomes more likely to provide a solution than this, then you change the focus. In every large team, when you are really trying to solve something unknown, you need enough freelancers around to explore possibilities around the major question. In many cases, that exploration is going outside and seeing if you can find a better idea across the street. This is true in managing research in companies: you put your team on your objective, but if you are clever, you have people whose business is to go around, sort of like a honey bee, and pollinate ideas and pick up pollen and make honey out of it, because you keep yourself open to new ideas, and frequently, that’s where the answer comes from.

MURJ: When you are talking about small teams, you said that context is really important. I’m wondering: in the larger question of context in the biological sciences, it seems like a lot of cell-culture based results don’t translate very well, which suggests that perhaps we should be looking at more levels of complexity when studying things that we hope to make as therapeutics. That’s a very hard problem and will be different for every situation. However, what kind of heuristics should we use to determine what context to look at a problem in, so that it is both experimentally viable and therapeutically translatable? What questions should we be asking there?

Sharp: The fundamental that’s universal to all science is, can you describe the experiment in a way such that, if somebody else in another room, not in contact with you, can read that experimental paper and design an experiment to reconstruct what you’ve done. The closer you can get to that in biology, the more powerful your science is. One of the things you just commented on is that as we grow tumor cells in culture, they change. If we are looking at experiments about how fast they migrate, whether they actually change shape, all these sorts of things, then that is very contextual. I worked for many years on tumor cells, but when I moved to trying to understand fundamental processes, I adapted the embryonic stem cell because the embryonic stem cell will proliferate forever, its diploid, its very similar if I go to Europe and
get an embryonic stem cell, I can take that cell and put in back into a blast in a mouse and generate a mouse. As long as I work with them, yes, there will still be contextual effects, but they should be less, and they are less, and there is more reproducibility. I can import data from different places and say, “I have a greater confidence this will be what I’ll see when I do the experiment,” and that’s why I moved to embryonic stem cells. However, your question is really wise, because we are moving now to a point where we can do much of our cell biology on one cell, or a few cells, and the faster we move toward organoid-type cultures, where we are looking at cells under more normal conditions, or even doing analysis directly on human tissue, primary tissue out of the individual, the more valid our science is going to be in understanding those disease states.

Sharp: We are in the midst of a big change in molecular biology. We are able to and required to quantitate more closely what’s going on, at bigger scales and in more complex systems. I was sitting here with a student just before you came in for this interview, talking about a certain process and trying to get more integration of different layers of regulation in different cells. So, yes. We are losing information when we do things “up, down, or no change,” but we are at a stage now when we have a lot more information than we know how to process. It’s complicated. It’s a network of interactions, and when you are parsing out bits of changes, this may contribute 5%, and this may contribute 10%, and this will be 50%, but they all are feeding back on each other in a very complex web. How to deal with this, and quantitate it and predict its behavior and focus on certain things is the challenge, really, of what we are doing, and its called systems biology. It isn’t simple, and its not going to be simple to answer. We are going to have to not only answer it in different ways, but abstract the answer in our language. Language is integral to doing science because it is the way we talk to each other: we use equations and we use language. In physics you can use equations more than you use language, although you have to have language too. You can use equations to formalize your language. In biology, molecular biology in particular, the equations frequently don’t give us a lot of predictive power, so you use a lot of descriptive language instead. But, we are going to have to evolve our language along with our predictive, computational power. We are at a community where students who are now being trained in biology are more quantitative than they were ten years ago or twenty years ago. They will, and others joining us (particularly I’m interested in the engineers joining), develop a new language as to the way we talk about this. We won’t talk about aging. We’ll talk about a system of genes. We won’t talk about plus-minuses. We’ll talk about feed-forward loops and incoherent and coherent changes. That will allow us to continue to abstract and get more powerful with broad concepts, but more specific in particular cases.

MURJ: In terms of abstracting: for systems biology now, as you apply more equations and formalisms, it gets very complex. Is there a point where that complexity stops being useful? Is there a stopping point where we should say, “OK, it’s about like this?”

Sharp: A model stops being useful when it exceeds what you can hold in your mind. Hopefully, with abstractions, we will devise more broad generalizations. Once you teach a generalization, then for every specific, you can move from the generalization to a specific and at least see that the specific is coherent with the generalization. You can then move to something more specific, where you won’t be able to use the generalization. I can give you an example. Chemistry 100 years ago was the reactivity of the periodic table. You understood that this reacted with A, but not with B because it stood here in the periodic chart. That was about it, and it turned yellow or blue. With the development of quantum
mechanics, molecular orbitals, and covalent bonds, we then developed an abstraction of chemistry that explained the periodic table, explained the sort of valency we observed. We could use this theory to predict, in somewhat quantitative ways, spectrum, reactivity, and whatever other properties. Where systems biology is going to take us is to the quantum understanding equivalent in biology. We’re not there yet. We haven’t seen Linus Pauling walk into the room, but we will find Linus Pauling walking into the room some time in the next twenty years, and he’ll appear, or she’ll appear in class, and she’ll start abstracting with higher-level thinking about how biological systems work. And she’ll create generalizations that allow students to say, “I can learn these four things and then I can understand microbes and mammalian cells and trees.” The textbooks are getting too big. You can’t learn anything out of a textbook anymore—it’s impossible. We’ve even given up giving them to you, or telling you to buy them. Go and read pieces of them online, it’s just as good as anything else. But there is a coherency to the whole science that can be taught.

MURJ: While we wait for the Linus Pauling or female equivalent, is there any way to systematically go about trying to abstract biology? Is there a set of experiments we should be doing? Or a way of thinking that we’re not yet thinking?

Sharp: Well, everyone is trying. Marc Kirschner at Harvard is doing some interesting things. Systems biologists here are as well. But it’s not going to be easy—it’s going to be fun, but it’s not going to be easy. That’s where we’re at. We’re very early in the evolution of the science.

MURJ: Taking a step back a little bit, the people you founded Biogen with are group of extraordinary people. Two of you ended up winning the Nobel Prize. How did you pick your colleagues?

Sharp: I picked them because they were extraordinary people. They were people you found interesting. They were people you found interesting and were real doers. You get to know those people. We, in any community—and that’s one of the pleasures of being a scientist, or a businessperson, or anything else that people do too—the community itself will recognize individuals who contribute and lead more than others. This can be mutual respect in a small community. They are invited to give presentations at meetings, they are invited to write reviews, and that sort of thing. They are the individuals who have the most novel idea. Within communities, you segregate out into people who are more concrete in what they enjoy talking about and people who are more “fluffy.” So, in this group, and it was an international group, they were all people we knew and they were people who had done significant things in this field, who were good people. They didn’t have egos so big they couldn’t work with others. And they self-selected in that, when they were presented with the opportunity, they wanted to join. And it was just fun. We got to know each other extraordinarily well, we traveled together, we did many things together, it was great fun. Wally Gilbert, forty years ago, was running around saying “some day I’ll have my genome sequence on this pen,” and they would laugh at him, but he’s got it on his pen now. Wally understood what was going on.

MURJ: When you were here at the Center for Cancer Research, you were here during what really was a revolutionary time. And certainly, Biogen has been this extremely productive place. So, you’ve interacted with places a lot where important things were getting done, and getting done very rapidly. (“It didn’t feel like rapidly when we were doing it!” Sharp interjects). What do you think makes places, communities, or institutions productive in that way? Is it just that people stumble on the right sort of ideas, is it that there are the right sort of social norms, or what?

Sharp: Really interesting people collect together. When I was describing Luria, he was an extraordinarily interesting person unto himself, but he was older than I was, and I wasn’t there when he was in his prime. I’m sure he was a charmer beyond what you can imagine, because I know the people who knew him and worked with him, Max Delbruck and Hershey, and these were all very different people, but they didn’t suffer fools at all. David Baltimore was just one of these incredibly charismatic, engaged, 100% involved people. He still is, he’s 75 now, he’s still very engaged. So, you want to be around someone like that, so you come, and you’re around them, and even when they’re really good, they’re open to anyone. To a student coming across the street, to something that happened across the country, they reach out immediately and say, “Oh, this is interesting, come and tell me about what you are doing.
and about how it works. Are there new ideas here? Is there something I can put into my mix? It was this very exciting environment to be in because there were leaders who were doing exciting things, and openness to new ideas and new people. And an immediate openness. Not being concerned about making a fool of yourself or taking risks because at the end of the day you know you can manage through it, and it’s going to be more important to find out what’s new.

This meritocratic environment we have here at MIT, in which everybody walks in the door, they know they are here because they were invited to be here, not because of money or because of fathers or mothers or anything else, it’s an unusual environment in which each individual sorts and forms their own communities and finds out what they want to do with their time here. That’s part of the faculty, it’s part of the heart of the place—that’s a power that very few places on earth have. You travel around and you find yourself in the middle of an Asian university, and it’s so formal, and it’s so focused on a subgroup of people, who have to be the subgroup of people who do the thing, and it just slows everything down and it’s just much less interesting. This is really a vibrant and unusual community, a special place.

**MURJ: The famous philosopher of science Thomas Kuhn said that science worked through revolutions or paradigm shifts where the context in which things are understood just completely shifts, very rapidly. Do you agree with that perception? Is that how science moves forward?**

**Sharp:** Yes. And, I’ve lived through two. I wasn’t there when DNA was discovered, I was seven years old, but I was there when recombinant DNA happened, and that was a discontinuity. It changed everything in our science after that. And genomics is a discontinuity. It’s big science, it’s being able to attack problems at a different level. What it means when something like that happens in the field is that if you don’t, individually, make the commitment to changing and becoming engaged in that discontinuity, you soon will not be interesting. And there was a parting of the way in both cases. It doesn’t mean you have to jump in front of it, but it means you have to take yourself to a place where you can use what’s going on and the new thoughts that come out of it in looking at problems that you’re interested in. So, we were looking through those two lenses of discontinuity, some conceptual, some technical, but really discontinuities. I see this continuing. Those were both 20 years apart. 20 years is a generation, so I see it continuing. And you’ll see patterns of similar changes in physics and in other sciences. And then there are these great discontinuities. Watson and Crick was a great discontinuity, one-in-a-century type. From Mendel. Darwin and Mendel happened at about the same time, and then we went another hundred years building on Darwin and Mendel until DNA, which took it to another granularity that Darwin and Mendel just weren’t able to think about. That’s where we’re at now, and a hundred years from now we’ll do something else. I think it will be just a great time.
The Characterization of Thin Film SnS for Solar Cell Applications

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Introduction

Solar cell research is very important in the ongoing process to move away from fossil fuels as a primary global energy source. Currently a great quantity of solar cell research works to optimize Si (silicon) solar cells. Si solar cells are solar cells with their primary electron generation components made of Silicon. Factors that affect how efficient these solar cells work include: thickness, different kinds of anti-reflective coating, and doping to make it more efficient. The relative abundance of Si makes it an attractive choice for solar cells. The primary issue with Si solar cells is they contain an indirect band gap, which means that to transfer an electron from valence to conduction bands a momentum term is also needed. This requires the Si solar panels to be produced upwards of 100 microns thick. As an alternative, some rare earth metal semi-conductors with direct band gaps are used to make solar cells. However the scarcity of metals like indium and tellurium in the earth’s crust put a great deal of strain on rare metal producers and the solar cell market would be heavily dependent on the few sources of these metals.

The research focuses on a lesser known material combination for solar cells in the form of SnS (tin sulfide). The SnS semi-conductor does not face the same problems as the rare earth semi-conductors because both tin and sulfur are relatively more abundant in the earth’s crust. Also, SnS has a direct band gap at 1.3 eV which is slightly larger than Si band gap of 1.1 eV but the electrons do not require a momentum term to shift into the conduction band because of the direct band gap structure. This would enable SnS solar cells to be much thinner than their Si counterparts and could be produced using continuous processing instead of batch processing.

Current record efficiency places the SnS solar cell’s efficiency at 4.0% however the verified published efficiency is 2% (Burton, Walsh, 2013). Theoretically the efficiency could be ~32% according to the Shockley-Queisser efficiency limit. The SnS itself has beneficial material properties including a high absorption coefficient of 105/cm, which is crucial for light absorption in a thin film. The majority carrier mobility has a believable mobility range in the 10s of cm²/Vs.

Methods

The work emphasizes the exploration of the structure of SnS. Using thin film samples of SnS (~0.1 to 1 micron thick) produced by thermal evaporation, the samples are analyzed using X-Ray diffraction (XRD) to determine what the crystalline structure is and how far the interatomic spacing is within the standard SnS orthomeric structure. The XRD quantifies the structural changes with the relationship of these variations with the density of states and band gap structure of the material (which ultimately affect conductivity, charge carrier mobility and quantum efficiency within a SnS solar cell). Scanning electron microscopy (SEM) is employed to observe the structure of the material and test the effects of the variables in the production process.

A majority of work pertains to the SEM images and modeling the relationship between the morphology of SnS grain structure and the annealing conditions the samples undergo. To accomplish this task, SnS that has been annealed in varying temperature and time conditions is characterized by SEM images. The last variable is the atmosphere of annealing which is either high in S₉ or low in S₉. Afterwards, the grain area distributions are represented graphically to determine...
a growth model which best represents the grain growth of SnS post processing. The grain growth has a strong correlation to material properties.

**Preliminary Data and Results**

![Histogram of Rice Grain Area](image1.png)

**Figure 1.** Histogram of typical lognormal distribution of the grain areas. This sample in particular was not annealed indicated by the large number of small grains present.

![300°C Vs. 400°C Cumulative Distribution Function](image2.png)

**Figure 2.** Cumulative Distribution Function of data comparing samples with two different annealing temperatures. The X axis represents the grain size, the y axis represents the probability of finding that grain size or smaller. The general trend is that functions closer towards the inside represent samples with relatively larger grains on average and functions closer towards the outside represent samples with relatively smaller grains on average. This figure tentatively shows that samples annealed at high temperatures for the same amount of time experience higher grain growth and have larger grains on average than samples that are annealed at lower temperatures for the same amount of time.

**Discussion and Future Direction**

The area distributions suggest an abnormal secondary grain growth model (Thompson, 2000). This is evident by the columnar initial grain structure of the SnS thin films, the texture of the film, and surface energy conditions which favor one grain growth over another. The graphs above are of the area distribution of the grains. The larger but less frequent grain size become more prevalent under longer annealing periods. Further data has to be taken to determine the effect of temperature of annealing and annealing atmospheric conditions.

This research will conclude with a compiled report on SnS structure and conclusive results about how the structure changes when SnS is fabricated in slightly different conditions. This research would support the larger branch of research under Katherine Hartman and Professor Tonio Buonassisi to help make thin film SnS solar cells a more feasible solar cell alternative to Si (Hartman, 2011).

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**References**


Characterizing MDSC Migration After Functionalization with Targeted Microparticles

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Introduction

Cancers are responsible for nearly 7.6 million deaths per year and unfortunately most leading chemotherapeutic treatments are highly toxic to healthy cells. In an attempt to combat this issue, controlled drug delivery systems are used in which drugs are transported to the site of the tumor, thereby limiting toxicity and dosage concentration (Srivasta et al. 2012). We propose to use myeloid derived suppressor cells (MDSCs) attached to polymer backpacks as a drug delivery vehicle to achieve targeted drug delivery to the tumor site.

MDSCs are found in bone marrow and are commonly known for suppression of the anti-tumor response and facilitation of tumor metastasis. Tumors overexpress colony stimulating factors such as GMCSF, MCSF, and IL-6 which in turn stimulate growth and development of MDSCs. In response to these chemokines, MDSCs migrate to the tumor site where they inhibit the anti-tumor immune response.

The aim of this investigation is to harness this tumor-specific tropism of MDSCs in order to deliver drugs directly to the tumor site. Microparticles are attached as drug-carrying backpacks onto MDSCs. The specific particles chosen for use in this investigation were layered polymers with a cell-adhesive region, a payload region, and a release region for efficient cellular attachment and drug release (Swiston et al. 2010). The end goal is to use MDSCs to transport the drug loaded backpacks to the site of the tumor and unload the therapeutic agent.

Methods and Results

CD11b+/Gr1+ MDSCs were isolated from spleens of tumor-bearing mice using magnetic-activated cell sorting (MACS) and the purity of the isolated MDSC population was evaluated using flow cytometry. MDSCs were stained with anti-CD11b FITC and anti-Gr1 PE antibodies and analyzed. Flow cytometry results showed that 99.4% of the MDSCs expressed both CD11b and Gr-1 (Figure 1). These MDSCs then were conjugated to rhodamine labeled polymer backpacks (Figure 2) and were observed through the use of fluorescence microscopy (Figure 3). MDSCs functionalized with backpacks were then run through a transwell polycarbonate membrane either toward control medium (IMDM) or tumor conditioned medium (TCCM) and the resultant migration was compared to control non-functionalized MDSCs. After the migration, the MDSCs that migrated through the transwell membrane were again observed under the fluorescent microscope. There were fewer MDSCs with attached backpacks as observed by the decreased red fluorescence observed post-migration (Figure 4).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{facs.png}
\caption{FACS results showing that the isolated MDSCs were positive for surface markers GR1 (PE) and CD11b (FITC)}\end{figure}
The migrated cells were then counted and these values were compared to the known amount of cells originally plated for the migration assay to calculate a percent migration. There was a significant difference between the percent of MDSCs with and without backpacks in TCCM and there was a large significant difference between the percent of migrated MDSCs in control versus tumor conditioned media in both MDSCs only and MDSCs with backpacks (Figure 5).

Conclusions and Future Direction

The results from the migration assay demonstrate that the tumor conditioned media performed far better than the control media (IMDM) in terms of attracting MDSC migration. However, there was a significant decrease in migration (~30%) in migration towards TCCM when backpacks were attached to MDSCs. This indicates that the backpacks are in fact impeding the motility of the MDSCs and affect tropism towards growth factors released by tumor cells. This could be caused by the backpacks either blocking chemical receptors on the MDSC surface or by the increased size of the MDSC-backpack complex. Future studies will investigate mechanisms through which migration and cellular viability can be improved both in-vitro and in-vivo.

Acknowledgements

I’d like to thank Darrell Irvine for allowing me to work in his lab, the Koch Institute for their wonderful facilities and openness to allowing undergraduate research and most importantly, my mentor Kavya Rakhra for being an incredible teacher and a devoted researcher. This work was conducted under Dr. Rakhra in conjunction with Rosanna Lim from the Rubner lab.

References

**MURJ – Georgia Tech Tower Collaboration**

In this special volume, the MIT Undergraduate Journal (*MURJ*) features a research report co-authored by students Helen Li and Zachary Pritchard, two undergraduates from the Georgia Institute of Technology. This article is featured as part of *MURJ*’s first-ever article exchange in collaboration with Georgia Tech’s The Tower Undergraduate Research Journal. An article titled “Synthesis of Polypeptides for Hydrogel Scaffolds” co-authored by MIT student Kiara Cui was published in Volume VI, Issue I of The Tower. Cui’s mentor Dr. Paula Hammond, David H. Koch Professor in Engineering, Department of Chemical Engineering, is a key member of the Emergent Behaviors of Integrated Cellular Systems (EBICS) center. EBICS is a NSF-funded science and technology center whose core institutions are MIT, Georgia Tech, and the University of Illinois at Urbana-Champaign. As such, Cui’s article was selected for the article exchange to highlight the increasingly collaborative nature of research in the modern age. We at *MURJ* hope that MIT and Georgia Tech will continue to have prospering relations in the future. See page 38 for the full report!

**Sigma Xi**

Sigma Xi is an international science honor society that promotes scientific research and recognizes the individual accomplishments of scientists, engineers, economists, and mathematicians. This prestigious organization espouses companionship amongst those involved with the scientific pursuit and publishes the magazine American Scientist. Sigma Xi has over 60,000 members in over 100 countries around the globe and 500 chapters. The MIT chapter is one of Sigma Xi’s oldest and largest chapters. Historically, the MIT chapter has been geared towards recruiting only highly accomplished seniors, graduate students, and postdoctoral fellows. At MIT, however, the vast majority of undergraduates engage in research projects through UROP and/or departmental incentives. The MIT chapter, therefore, has decided to change its focus to undergraduate students and reorganize into an undergraduate-centered chapter. This newly organized undergraduate-centered chapter will be partnering with MURJ to actively engage the large population of active undergraduate researchers. In fact, members of this new chapter helped MURJ run its first annual CPW Poster Competition. Participants of the MURJ-Sigma Xi CPW Poster Competition will all be invited to join this new Sigma Xi Chapter.
Characterizing the Effect of Inactivating SIRT1 in Mouse Embryonic Stem Cells

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Embryonic stem (ES) cells have come to be recognized for their potential role in regenerative medicine. Since the discovery of induced pluripotent stem cells (iPSCs) in 2006, there has been an ongoing effort to improve the process of reprogramming differentiated cells. Improved understanding of pluripotency pathways will enable researchers to overcome the current barriers to efficient and safe reprogramming. The activation of SIRT1, an NAD-dependent deacetylase, has recently been found to improve efficacy of iPSC reprogramming. A member of the family of sirtuins, SIRT1 has been linked to the aging process and is localized to euchromatin in the nucleus. Here, we show that in the absence of catalytically active SIRT1, mouse embryonic stem (mES) cells proliferate at slower rates as compared to wild type cells. The reduction in proliferation is cell autonomous and is linked to a set of genetic changes induced by the knockout of SIRT1. Additionally, when SIRT1 is inactivated, mES cells adopt an epiblast stem cell phenotype that is indicative of a more differentiated form of pluripotency termed “primed” pluripotency. Thus, our findings implicate a pluripotency pathway that is mediated by SIRT1, knowledge of which could serve to improve techniques used to more efficiently and safely create iPSCs.

Introduction

Embryonic stem (ES) cells are unique in their ability to give rise to any cell type. For this reason, ES cells have been identified as potential therapeutic targets for a wide range of pathologies. However, current setbacks have prevented an effective translation of ES cells for medical use. For example, ES cells that have been directly derived from embryos are limited by availability and their use has also been contested due to ethical concerns. A potential solution to this problem was identified in 2006 with the advent of induced pluripotent stem cells (iPSCs). Shinya Yamanaka of Kyoto University demonstrated the capacity of differentiated adult cells to be reprogrammed to pluripotent stem cells by transfecting the adult cells to introduce four genes: Oct4, Sox2, Klf4, and Myc (Takahashi and Yamanaka, 2006). Currently, iPSCs are not approved for medical application since the method of reprogramming adult cells to iPSCs is both inefficient and too risky to be safely used as a treatment.

Viral reprogramming is one of the more effective methods of creating iPSCs, however, this method is not recommended for use in patients. Furthermore, reprogramming efficiency is often extremely low, in the range of 0.01-1% (Rais et al., 2013). Therefore, an improved method of reprogramming is currently being sought to enable implementation of iPSCs in medical therapies. It is much simpler to convert ES cells to adult cells than it is to do the reverse. An ES cell can simply be exposed to differentiation factors such as Fgf or activin and will differentiate, however chemicals that lead to the induction of pluripotent stem cells from adult cells without necessitating direct genetic activation have yet to be identified (Nichols and Smith, 2009). Thus, improved understanding of pluripotency pathways could enable researchers to identify a new method by which human cells can be manipulated for medical use.

It was recently discovered that SIRT1 stimulates the formation of iPSCs via a SIRT1-p53 pathway (Lee et al., 2012). SIRT1 is a member of the family of sirtuins, which have been previously associated with aging (Michan and Sinclair, 2007). The aforementioned study demonstrated that knockdown of SIRT1 decreased the efficiency of iPSC formation, whereas the treatment of wild type cells with resveratrol, a known SIRT1 activator, led to an increase in iPSC formation efficiency (Lee et al., 2012).
To further corroborate such findings, Lee et al. (2012) also overexpressed SIRT1 in cells and found that reprogramming efficiency was again increased in this instance. Based on these findings, we set out to explore the role of SIRT1 in ES cells. Here, we propose that in the absence of SIRT1, differentiated cells are more resistant to reprogramming, and that SIRT1 may play a role in the maintenance of naïve pluripotency.

Materials and Methods

**mES Cell Culture**

mES cell lines 30, 31, 33, 34, 36, 37, 38, T6-11, and T6-13 were derived from 3-day-old mouse blastocysts using transgenic mice from Jax (strain numbers 004682, 008041, and 007676). Lines were maintained on irradiated feeder MEF cells (TPA E13.5 mice, Jax #002508; 50,000/cm²) in filtered Embryomax Dulbecco’s modified Eagle’s media (Millipore) supplemented with nonessential amino acids, 15% ES qualified fetal bovine serum, glutamate, antibiotic antimycotic (Penicillin, Streptomycin, and Ampicillin), LIF, and 2-ME. 20,000 mES cells/cm² were plated onto the previously described gelatinized plates and appropriate lines were treated with 1μM Tamoxifen for 24 hours prior to the start of experiments to delete exon 4 of SIRT1. MEFs were derived from pups that were dissected and treated with 0.25% trypsin for 30 min and then plated and incubated at 37°C.

**Naïve to Prime Differentiation**

mES cell lines 33,34,36,37,T6-11, and T6-13 (+/- tamoxifen) were plated in differentiation media for 3 passages in the absence of MEFs. Differentiation media contained N2B27 media (50% Neurobasal/50% DMEM/F12, 2% B27 supplement, 1% N2 supplement, 0.875% BSA, 2-ME, and antibiotic antimycotic) supplemented with 20ng/ml Activin A and 12ng/ml of FGF2. After the initial 3 passages, primed cells were maintained in differentiation media with irradiated MEFs.

**Prime to Naïve Reprogramming**

Primed cell lines 33,34,36,37,T6-11, and T6-13 (+/- tamoxifen) were transferred to reprogramming media with irradiated MEFs for 5 passages. Reprogramming media contained Ham/s F12, 20% Knockout Serum Replacement, nonessential amino acids, 2-ME, LIF, and antibiotic antimycotic.

**Clonogenic Assay**

Cells were trypsinized to a single cell suspension and counted. 50,000 cells were plated in ES media and grown for 3 days. Cells were fixed in 4% PFA for 15 minutes and washed 3 times with alkaline phosphatase buffer (0.1M Tris pH 9.5, 0.1M NaCl,0.05M MgCl2). Cells were incubated overnight at 4°C in alkaline phosphatase buffer supplement with 5ug/ml nitro-blue tetrazolium chloride (NBT) and 2.5ug/ml 5-bromo-4-chloro-3’-indolylphosphate p-toluidine salt (BCIP). Alkaline phosphatase positive colonies were counted using Zeiss microscope.

**Microscopy**

Cells were plated on 25mm circular coverslips in 6-well plates for 2 days, and then fixed in 4% paraformaldehyde for 15 minutes at 25°C. Antigen retrieval was performed using 0.25% TritonX-100 for 10 minutes at 25°C. Cells were then washed in PBS and incubated in Blocking Reagent (#FP1012, Perkin Elmer, Akron, OH) with 5% horse inactivated goat serum for 30 minutes at 25°C. Cells were incubated overnight at 4°C in primary antibody (SIRT1 # 2028, H3k4me3 # 9727, Cell Signaling Technology, Danvers, MA; Oct4, #5279, Santa Cruz, Dallas, TX), washed, and incubated in corresponding secondary antibody for 1 hour at 25°C. Cells were stained in DAPI for 5 minutes at 25°C and mounted using Fluoromount-G (Southern Biotech, Birmingham, AL). Slides were imaged using either Confocal Microscope at 60X or Zeiss Microscope at 5X or 20X.

**RNA Isolation and Sequencing**

RNA was isolated from mES cells following the protocol for total RNA purification from cultured cells and tissue with NucleoSpin® RNAII as described in the Total RNA isolation kit from Macherey-Nagel (Bethlehem, PA). RNA isolates were sent to the BioMicro Center at MIT (Cambridge, MA) to be sequenced. mRNA was prepared using strand-specific Ribo-Zero kit (Illumina, Madison, WI) to remove rRNA. The sequencing utilized an Illumina HiSeq platform, and the read length was 40 nucleotides. RNA Sequencing data was processed using the BMC/BCC 1.0.2 pipeline. Data were then normalized and analyzed using the Cufflinks program by Galaxy.

**Results**

**SIRT1 Localized to Nucleus in Actively Transcribed Regions**

To better understand the role of SIRT1 in ES cells, we began by using antibody binding and microscopy to localize SIRT1 within ES cells. It was found that SIRT1 is expressed in the nucleus, as confocal imaging of the nuclear fraction revealed that SIRT1 is expressed in the same regions that showed positive staining for
DAPI, which is known to stain the nucleus (Fig. 1). To further assess the localization of SIRT1, we also applied antibodies against H3K4me3; an activating mark in the cell that is typically associated with euchromatin and is therefore indicative of sites of active transcription. Confocal microscopy revealed that SIRT1 is expressed in the actively transcribed nuclear fraction as the signal for SIRT1 overlaps with the signal for H3K4me3 (Fig. 1).

SIRT1 Null ES cell exhibited no signal using SIRT1 antibody, indicating specificity of the SIRT1 antibody (Fig. 1B). Because SIRT1 is thought to be involved in pluripotency, we also imaged cells to determine whether SIRT1 is co-expressed with Oct4, a key marker of pluripotency. It was found that SIRT1 is co-expressed with Oct4, and DAPI staining corroborated the results from Figure 1 indicating that SIRT1 is localized to the nucleus (Fig. 2).

Inactivation of SIRT1 in mES cells Reduces Cell Count in a Cell Autonomous Manner

To better characterize the role of SIRT1 in ES cells, we studied the proliferation of SIRT1 knockout (KO) cells as compared to wildtype (WT). We added tamoxifen or DMSO to an equal number of cells at day zero and counted the number of cells over time. Cell counts of WT to KO cell lines were compared to quantify the differences in proliferation. SIRT1 knockout cells were generated by introducing tamoxifen to cells containing a transgene for a CreERT and loxP sites flanking exon 4 of SIRT1 (Lines 34 and 36). Tamoxifen was chosen for treatment because it induces proper folding of CreERT when administered to cells such that upon treatment with tamoxifen, Cre is able enter the nucleus to interact with the loxP sites used in our transgenes.

Figure 1. SIRT1 is expressed in the actively transcribed nuclear fraction. ES Cells were fixed and probed for SIRT1 and H3K4me3. Confocal microscopy at 60X magnification was used to image the cells. DAPI staining is represented by blue signal, H3K4me3 by green, and SIRT1 by red. Scale is 5μm.

Figure 2. SIRT1 is co-expressed with Oct4. ES WT and SIRT1 Null cells were probed for Oct4 and SIRT1. Top three rows were imaged at 20X magnification; bottom row was imaged at 5X magnification. DAPI staining is represented by blue signal, Oct4 by red, and SIRT1 by green. Scale is 5μm for top 3 rows and 63μm for bottom row.
The WT cell lines were 30 and 31, which did not contain the CreERT transgene, were non-inducible because there was no genetic alteration of SIRT1 activity in these cells when given tamoxifen since these cells lacked inducible Cre. The mES cells also contain a transgene that encodes for a tomato reporter, followed by a stop site and then a GFP reporter, such that the cells typically fluoresce red when not exposed to Cre recombinase. When cells with inducible Cre are exposed to tamoxifen, however, the loxP sites of the transgene recombine and both the stop site and the tomato reporter are removed so that the cells fluoresce green instead. Therefore, this shift from red to green is also indicative of the knockout genotype that is induced when tamoxifen is given to the inducible cells.

mES cells with the SIRT1 KO phenotype were observed to be less abundant and did not populate plates in cell culture as rapidly as WT mES cells. To better quantify this difference in cell count over time, we conducted an assay, which showed that by 15 days into the experiment, cell counts for SIRT1 KO mES cells were significantly lower than those of the WT mES cells (Fig. 3A). To determine whether this effect was cell autonomous, or due to extracellular signaling occurring in the media, we designed an assay combining equal volumes of SIRT1 KO and WT cells and passaged them over time to determine whether either cell type would outcompete the other. The control for this assay was a combination of non-inducible WT mES cells that were either untreated or treated with tamoxifen. The results of this assay demonstrate that WT mES cells outcompete SIRT1 KO mES cells, indicating that the inactivation of SIRT1 reduced the cell count of SIRT1 KO cell numbers in a cell autonomous fashion (Fig. 3B).

We hypothesized that the inactivation of SIRT1 may be inducing transcriptional changes that lead the mES cells to differentiate, therefore causing them to grow more slowly and senesce. We hypothesized that SIRT1 may be playing a role in the maintenance of pluripotency; however, further characterization of SIRT1’s role in pluripotency has yet to be elucidated. To assess transcriptional changes in the KO lines, samples of RNA isolated from mES cell lines 31, 31T, 34, (all WT) and 34T (KO) were each sequenced in triplicate. Line 31T was used as a measure of the effect of tamoxifen on the transcriptional expression in cells, whilst line 34T was used to assess the effect of SIRT1 inactivation on transcription. Analysis of the data obtained from the RNAseq revealed that the expression of several genes involved in pluripotency pathways had been altered in the absence of functional SIRT1 (Fig. 4A).

Further investigation of these pathways led us to realize that many of the changes in the KO were to the expression levels of factors involved in primed pluripotency networks such as Fgf2, TGF-β, and SMAD. These changes bore resemblance to the transcriptional changes in epiblast stem cells that were previously studied by Kim et al (2013). Comparison of the upstream analysis of SIRT1 KO mES cells and epiblast stem cells revealed that several of the same regulators had been either activated or inhibited in a statistically significant manner (Fig. 4A).

In response to this finding, we compared the number of genes that changed in the same direction (either
activation or inhibition) with statistical significance between WT mES cells and Epiblast stem cells, or between SIRT1 KO mES cells and the SIRT1 WT mES cells. This comparison revealed that SIRT1 KO mES cells shared a statistically significant amount (p = 2.71 e-44) of activated or inhibited gene expression changes (Fig. 4B). As a control, transcriptional expression changes observed between lines 31 and 31T were also compared to changes amongst ES and epiblast stem cells. Since line 31T is still WT, this comparison evaluated the effect of adding tamoxifen to the cells. No statistically significant similarity was revealed between changes induced in line 31T as compared to those observed in epiblasts (Fig. 4B). These results indicate that SIRT1 likely plays a role in the maintenance of pluripotency networks in stem cells, and that in the absence of SIRT1, ES cells begin to differentiate in culture.

**SIRT1 KO ES Cells Differentiate More Efficiently to Primed Pluripotent State**

Based on the data we gathered from RNaseq, we believe that SIRT1 may play a role in the transition from naïve to prime state pluripotency. To further investigate this idea, we performed a clonogenic assay. Naïve cells are clonogenic whereas primed cells differentiate or undergo apoptosis when cultured as single cells. Our data show that SIRT1 KO cells lose clonogenicity more quickly than WT cells when in differentiation media; whereas WT cells with active SIRT1 maintain clonogenicity even when in differentiation media (Fig. 5 A&B).

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**Figure 4.** SIRT1 KO mES cells display a similar transcriptional profile as epiblast stem cells. (A) Comparison of upstream analysis of RNAseq data. (On Left) Upstream analysis of RNAseq data from SIRT1 KO mES cell lines 31, 31T, 34, and 34T was conducted using the software Ingenuity Interactive Pathway Analysis. Raw data for the ES/Epiblast comparison was obtained from Kim et al., 2013 and processed using Ingenuity Interactive Pathway Analysis software (On right). Indicated in red are genes that display similar changes in regulation in both the KO mES cells and the epiblast stem cells. (B) Comparison of number of genes that exhibit the same activation state with statistical significance. Top diagram is a comparison between epiblast stem cells and SIRT1 KO cells. Bottom diagram is a comparison between epiblast stem cells and WT cells. Epiblast stem cells and SIRT1 KO cells display greater similarity than do epiblast and WT.
Our data led us to then hypothesize that SIRT1 may play a role in maintaining ES cells in the naïve state, and so we conducted a second clonogenic assay to assess the effect of inactivating SIRT1 when reprogramming from the prime to naïve states. Previously passaged primed cells were plated in KOSR media with LIF on MEFs to reprogram the cells back to the naïve state. Tamoxifen was used to induce knockout after the cells differentiated to the primed state. Alkaline phosphatase staining revealed that in the absence of active SIRT1, primed cells were not as efficiently reprogrammed to the naïve state, suggesting that SIRT1 may play a role in this transition (Fig. 6 A&B).

**Figure 6.** SIRT1 KO cells are more resistant to prime to naïve reprogramming. (A) Inducible SIRT1 KO cell line 37 is a representative cell line used in this assay. DMSO treated cells are WT (blue) and tamoxifen treated cells are KO (red). (B) Clonogenic assay for naïve to prime differentiation. Two WT lines (blue) and four inducible KO lines (red) were evaluated for clonogenicity; passages 3 and 5 are shown here. Colonies were counted using alkaline phosphatase staining. Ratio of tamoxifen treated to DMSO treated cells is displayed and used for comparison. p<0.05
Discussion

Given that Oct4 expression is a key property of naïve pluripotency, our results suggest that SIRT1 may play a role in pluripotency since it is co-expressed with Oct4 (Fig. 2). Further examination of RNA sequencing data revealed that the altered expression of genes involved in pluripotency and development pathways strongly resembles the transcriptional expression pattern of epiblast stem cells as described by Kim et al., 2013 (Fig. 4A). We found that a statistically significant amount (p= 2.71 e-44) of transcriptional changes that occur in the transition from ES to epiblast stem cells also occur in the same direction from WT ES cells to our SIRT1 KO cells (Fig. 4B). This led us to wonder whether SIRT1 inactivation induces a phenotype that is very similar to the previously reported epiblast phenotype.

Epiblasts are notably different from ES cells due to differences in several properties involved in pluripotency and self-renewal. Epiblasts are considered primed cells, rather than naïve, as they are less capable of self-renewal and are somewhat more differentiated (Nichols and Smith, 2009). Furthermore, ES cells display round morphology while epiblast cells are flat (Nichols and Smith, 2009). Epiblasts also display low clonogenicity, which is significant in the field of iPSCs because clonogenicity is a measure of the degree to which a cell can be cultured as single cells (Nichols and Smith, 2009). It has proven difficult for scientists to obtain naïve stem cells from differentiated human cells, and so most human iPSCs exist in the primed state. However, it appears that naïve stem cells self-renew much more readily and therefore would increase the efficiency of iPSC generation and be more applicable to medical use (Gafni et al., 2013). Thus, identification of pathways involved in the maintenance of the naïve stem cell state would be significant to understanding and manipulating pluripotency pathways so as to more effectively generate naïve state human iPSCs.

Our data indicate that SIRT1 may be blocking the transition to a primed pluripotency network, or helping to maintain ES cells in the naïve state. The results of our clonogenicity assays show that SIRT1 KO cells lose clonogenicity over time, suggesting that these KO cells more readily differentiate to the primed state (Fig. 5&6). Furthermore, when primed SIRT1 KO cells were cultured in reprogramming media, these cells were more resistant to reprogramming and formed fewer colonies as compared to the WT cells (Fig.6). This reveals that SIRT1 may be involved in the transition from prime to naïve and that it may also be necessary to efficiently reprogram from the primed state to the naïve state.

Our finding that SIRT1 KO cells begin to adopt an epiblast transcriptional profile indicates that SIRT1 may be a part of a pluripotency pathway that could be manipulated to enhance the efficacy of the reprogramming of adult cells to iPSCs. Furthermore, our results that indicate that SIRT1 may be necessary to efficient reprogramming also warrant further research in the requirement for SIRT1 in this transition. One of the current issues with iPSCs is that they are still most effectively reprogrammed via transfection, which prevents their use as treatments. However, by further understanding pluripotency pathways, there is the potential that these pathways could be manipulated by alternative means that avoid the use of viruses. Should SIRT1 be confirmed to play an essential role in the reprogramming of differentiated cells; there are already many known specific SIRT1 activators such as resveratrol, SRT1720, or SRT2183, which could be used in reprogramming without use of a virus.

References

Cross-Linking of a Positive-Tone, Polynorbornene Dielectric

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Dielectric films are often used in microelectromechanical systems (MEMS) and microelectronics packaging, where good mechanical and electrical properties are desired to improve device processability and reliability. The good mechanical properties are achieved by addition of an epoxy cross-linker, which forms covalent bonds between polymer chains (cross-links) at elevated temperatures. In this study, cross-linking in a positive tone, aqueous-developable, polynorbornene (PNB) dielectric was investigated. Cross-linker loading and cure temperature were varied and their effects on residual stress and solvent swelling were measured. Residual stress in the film was confirmed to increase during the cure step, as expected. Additionally, residual stress and swelling were not affected by either cross-linker loading or cure temperature in the final film. This suggests that the maximum degree of cross-linking was obtained. Residual stress was, however, affected by cross-linker loading during some processing steps: films with lower cross-linker loadings absorbed more base during development and experienced higher stresses. This led to problems with film reliability since high-stress films tend to break apart easily. As a result, increasing cross-linker loading improves processability of the film even though the final properties are not affected.

Introduction

Dielectric films, which act as permanent insulators, are widely used in the chip packaging of microelectromechanical systems (MEMS) and microelectronic devices (Maier, 2001). These films serve to mechanically protect and electrically insulate active components, and thus are required to have high hardness and low electrical permittivity. Polymers can offer several advantages over inorganic compounds due to their relative toughness and less costly methods of processing (Mueller, 2012). In particular, the polymer known as polynorbornene (PNB) has low moisture uptake and a low dielectric constant (i.e., permittivity) due to its saturated hydrocarbon backbone, as shown in Figure 1 (Raeis-Zadeh & Kohl, 2012). This low permittivity is attractive because it would reduce electrical losses between active components and result in more energy-efficient devices (Mueller, 2012).

In addition, PNB can be photo-defined with the addition of a photo-active compound, diazonaphthoquinone (DNQ). A directly photo-definable dielectric film is attractive, because of its ease of processing (Mueller, 2012). The photo-definition of PNB is performed by exposing the film to ultraviolet light through a photomask. This photo-reaction of DNQ in the exposed regions causes a solubility increase in the developer, an aqueous base. Upon developing, the regions exposed to light dissolve away and the unexposed regions remain, resulting in a positive image transfer of the photomask (Raeis-Zadeh, Melendez, Chen, & Kohl, 2011). These dielectrics are thus termed “positive tone” and are desired for the selective creation of holes. Positive tone dielectrics were considered because they are more suitable for thick film dielectrics due to higher yield as a result of fewer photochemical reactions and mask type (i.e., less dust contamination) (Mueller, 2012). An additional benefit of the films studied is that the aqueous base developer used in processing is more environmentally friendly than solvent-based developers (Mueller, 2012).

With the addition of an epoxy cross-linker, the photo-definable PNB acts as a thermoset, creating a rigid three-dimensional structure after being cured in a nitrogen-purged oven at a specified cure temperature (Rajarathinam et al., 2009). The cure process is used to permanently set the film structure via cross-linking,
the formation of numerous covalent bonds between polymer chains, which is accomplished by elevating the temperature of the film in an oven. The cross-linker that was used, trimethylolpropane triglycidylether (TMPTGE), has three epoxide groups that readily cross-link to the PNB, as shown in Figure 2 (Raeis-Zadeh, Elce, Knapp, & Kohl, 2011).

The purpose of this research was to improve the mechanical properties of a photo-definable, positive tone, PNB dielectric film. These properties are significantly affected by the degree of cross-linking in the dielectric film. Specifically, the effects of cross-linker loading (i.e., the amount TMPTGE added) and cure temperature on the degree of cross-linking, and thus the mechanical properties of PNB, were investigated. It was hypothesized that increases in cross-linker loading and cure temperature would both contribute to a higher degree of cross-linking, as a higher loading allows more cross-linking to occur and higher cure temperatures provide more energy to fuel the cross-linking reaction. At a certain point, all cross-linking sites within the polymer that can reasonably react will have done so and the degree of cross-linking will plateau.

Methods

Materials and Formulations

A formulation must first be created with the following components: the polymer PNB, the photoactive compound DNQ, the cross-linker TMPTGE, and the solvent propylene glycol monomethyl ether acetate (PGMEA). The proportions of each component in the formulation were 30 wt% PNB in PGMEA, 20 mass parts DNQ to 100 mass parts PNB (pphr), and a varied amount of TMPTGE. PNB, as the polymer, forms the backbone of the film structure and DNQ gives the film its positive tone, photo-definable properties. Also, DNQ, the photoactive compound, was found to act as a cross-linker under certain conditions.

Film Creation

Thin film creation was accomplished by a five-step processing method, as shown in Figure 3:

1. Spin-coat: A small amount of the formulation was placed onto the center of a silicon wafer (100 mm diameter) and distributed via spin-coating on the CEE 100CB spinner at 2500 rpm for 60 seconds. At this point, the solution was evenly spread over the surface of the wafer, creating a thin film with a thickness of approximately 10 micrometers (µm).
2. Bake: The sample was placed onto a hot plate at 100 °C and heated for 120 seconds in order to harden the film and remove residual casting solvent.
3. Expose: The sample was exposed to ultraviolet light via an Oriel Instruments flood exposure tool, leaving a latent image visible on the film.
4. Develop: Using a 0.26N tetramethylammonium hydroxide developer, the latent image in the polymer was developed for 120 seconds and then rinsed with de-ionized water.
5. Cure: To achieve hardness and the final desirable properties, the wafer was cured in a nitrogen-purged furnace for 2 hours at the specified cure temperature.
Experiments for Cross-Linker Loading and Cure Temperature

To observe the effects of cross-linker loading and cure temperature on the degree of cross-linking, and thus the mechanical properties of PNB, three sets of samples were prepared. The first two sets were cured at 220°C and 180°C. Each of these sets consisted of formulations with varied cross-linker loading values: 0.0, 2.5, 5.0, 7.5, and 10 pphr. The third set of samples, however, had a constant cross-linker loading value of 5 pphr while the cure temperature was varied. Temperatures in the range 140 – 240 °C have been considered in research of negative tone PNB; thus, the third set of samples was cured at temperatures of 160, 180, 200, and 220°C (Raeis-Zadeh, Elce, Knapp, & Kohl, 2011).

Mechanical Properties Tested

After the creation of the film, the effects of cross-linker loading and cure temperature on the mechanical properties of each set of samples were measured, specifically the residual stress and solvent swelling. High residual stress within the film increases the chances of tearing and breaking; thus, lower stress is desired. As the degree of cross-linking increases, the ability of the film to swell with solvent is decreased. Thus, increased swelling indicates lower levels of cross-linking within the film.

To determine the stress, deflection measurements were recorded between each processing step by the Flexus, as illustrated in Figure 4.

The Flexus uses a laser of wavelength 670 nm to measure the radius of curvature for a wafer and then calculate the residual stress. The stress is determined from Eq. (1):

$$\sigma = \frac{E}{1-v} \frac{h^2}{6Rt}$$

where \(E/(1-v)\) is the elastic modulus of the substrate (1.805 \times 10^{11} \text{ Pa}) for <100> oriented silicon, \(h\) is the substrate thickness, and \(t\) is the thickness of the film. \(R\) is the change in radius, given by \(1/R = 1/R_1 - 1/R_2\), where \(R_1\) is the radius of curvature of the uncoated substrate and \(R_2\) is the radius of curvature of the substrate after film coating and processing.

Finally, the effects of swelling on the film are observed by submerging the wafers in PGMEA solvent for 15 minutes and recording the mass change. The percent of swelling, i.e., the relative amount of solvent uptake by the film, is measured by dividing the change in mass by the initial mass of the sample.

Results

At various point throughout processing, film stress was measured using deflection measurements obtained from the Flexus. Stress measurements after the development and cure steps are depicted in Figure 5 for various cross-linker loadings and a cure temperature of 220 °C. As shown, large increases in stress occur during the cure step, by as much as 20 MPa in the case of the 10 pphr sample.

Solvent swelling was measured in PGMEA for fully processed films both while cure temperature was varied (Figure 6) and while cross-linker loading was varied (Figure 7). Films with cross-linker loadings of 0.0 and 2.5 pphr were prepared, but did not survive swelling measurements, thus values are not reported.

Discussion

Figure 5 compares stress after development and curing for films of varying cross-linker loading. The increase in residual stress during the cure step results from exposure of the film to higher temperatures: during the cure process, remaining solvent and water are evaporated and the cross-linking reaction in the film is completed with energy supplied by the elevated temperature. These lead to a more rigid film after curing, which increases residual stress.
The stress after develop can be seen to vary with cross-linker loading; however after the cure step, this dependence is removed, with residual stress becoming independent of cross-linker loading. Stress after curing was also found to be independent of temperature.

Swelling is generally considered an indicator of the extent of cross-linking within a film: more densely cross-linked films are less capable of holding solvent and do not swell as much as films with less cross-linking. As with stress, there is no clear correlation between swelling and cure temperature (Figure 6) or cross-linker loading (Figure 7). The results for stress and swelling together suggest that for the reported temperatures and cross-linker loadings, the maximum degree of cross-linking was achieved—that is, the amount of cross-linking in the final film is not affected by the cure temperature or cross-linker loading.

It is worth noting that the maximum degree of cross-linking does not necessarily correspond to reaction at all possible cross-linking sites; if this was the case, there would be more cross-linking at higher cross-linker loadings than at lower ones.

Instead, cross-linking is limited by packing and access to reactive sites and only a fraction of the potential links are made. Once this critical amount of cross-linking is reached, addition of more TMPTGE does not affect the properties of the final film.

Excess cross-linker can still, however, have an effect in earlier processing steps, as suggested by Figure 5(a). TMPTGE in the formulation has the additional benefit of decreasing film solubility during processing, which can be helpful due to the fragile nature of these films. Films with low cross-linker loadings were consistently difficult to keep intact throughout the film creation and testing process, and data for 0.0 and 2.5 pphr is not reported in Figure 7 because these samples did not survive processing.

Figure 5. Film stress measurements for a constant cure temperature of 220 °C and varied cross-linker loadings after two processing steps: (a) development and (b) cure.

Figure 6. Base swelling in PGMEA. Lines represent different cure temperatures of 180, 200, and 200 °C with a constant cross-linker loading of 5.0 pphr.

Figure 7. Base swelling in PGMEA. Lines represent different cross-linker loadings of 5.0, 7.5, and 10 pphr with a constant cure temperature of 180 °C.
Future Work

To further the understanding of the cross-linking mechanism, future work will also measure the effect of cross-linker loading on electrical properties of the dielectric film, such as the dielectric constant, in addition to other mechanical properties, such as the elastic modulus and hardness. Because cross-linking can also be affected by the presence or absence of a post-develop exposure during the creation of the dielectric film, the influence of post-develop exposure should also be investigated. Moreover, this positive tone dielectric film will be compared to negative tone dielectric films. A strong acid is used for negative tone photo-definability, and this lowered pH may have large effect on film processing and properties.

References

Biomechanics Effects of Running on Load Distribution to the Plantar Surface

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Lower extremity injuries may be related to impact loads, which are affected by landing style, running shoe choice, terrain, and speed. In this study, the subject ran trials with varying shoe, surface, and speed conditions using a forefoot and then a heel strike landing style. In-shoe measurements of load imparted to the ball, arch, and heel were made using force sensing resistors. Although the observed running speed trends were small, our results indicate that loading – and potentially injury risk – can be more effectively reduced by adopting a forefoot strike, which reduces total loading from that of heel striking by almost 29%. Furthermore, running on dirt or grass instead of asphalt was found to decrease loading by 15% for heel striking and 16% for forefoot striking. Highly supportive shoes led to impact force reductions of nearly 26% and 36% for heel and forefoot striking, respectively, as compared to less supportive shoes.

Introduction

More than 35 million United States residents were estimated by American Sports Data, Inc (ASD), to have run at least once in 2002. As a testament to the long-term pull of the sport, the ASD also estimates that over 11 million runners have been running for 10 years or more (Long Distance Running – State of the Sport). Running is both a recreational and a fitness activity that requires relatively little gear and few expenses, but that yields beneficial increases in fitness and overall health; however, previous studies of running-related injuries report lower extremity injury rates ranging from 19.4% to 92.4% (Gent et al., 2007).

Many methods have been suggested to reduce the incidence of injury: landing on the forefoot or midfoot rather than the heel while running, running in more supportive or less supportive shoes, and running on softer terrain, among others. Running shoe companies began introducing more cushioned models around the 1970s, but in recent years a minimalist running movement has taken hold. Along with the minimalist movement has come the view that a forefoot strike, which is adopted more naturally by barefoot runners, increases efficiency and decreases injury risk (McDougall, 2011).

This experiment was designed to address widely held beliefs about methods of reducing injury risk through reductions in loading to the foot. A long-time heel-striker who converted to a forefoot strike was recruited to study the effects of changing one’s landing style on the loading distribution to the foot. Running trials were conducted under various running shoe, speed, and terrain conditions for both forefoot and heel striking, and results were compared with those of prior investigations. Most previous studies have considered loading to the foot as a whole, but this study compared loads imparted to the forefoot, arch, and heel using force pads inserted into the shoe.

Running Kinematics and Load Considerations

Foot strike describes the moment in a runner’s stride when the foot collides with the ground. In the case of a heel strike, which is practiced by some 75% of elite international runners, the heel collides first, followed by the forefoot (Hasegawa et al., 2007). A forefoot strike is characterized by the ball of the foot landing first, followed by the heel. Thus, since push-off at the end of the strike occurs from the forefoot, forefoot striking is often referred to as “toe-heel-toe” running, while heel striking is termed “heel-toe” running. Midfoot striking is an intermediate classification marked by the heel and ball of the foot landing simultaneously. These three classifications fall in a continuum of landing styles where forefoot and heel striking are the extremes, and midfoot striking is intermediate between the two.
striking comes somewhere in between (Lieberman et al., Biomechanics of Foot Strikes & Applications to Running Barefoot or in Minimal Footwear).

The loading to the foot upon impact is governed by Newton’s 2nd law:

\[ F = ma \]

where \( F \) is the impact force, \( m \) is the portion of the body that comes to a halt during impact (the effective mass), and \( a \) is the acceleration of the effective mass. Forefoot and heel striking generate different loading patterns due to their differences in effective mass and the conversion of vertical momentum. From studies of barefoot runners, the effective mass for forefoot striking was calculated to be approximately 1.7% of the total body mass, compared to about 6.8% for heel striking. These calculated differences in effective mass can be attributed to the conversion of vertical momentum upon impact. Most of the vertical momentum of the falling lower leg in a forefoot strike is converted into rotational momentum, lowering the effective mass. In contrast, heel striking results in much of the vertical momentum being absorbed and contributing to the impact force, so the effective mass is much higher. Due to these kinematic differences, heel-striking runners suffer an impact transient – an abrupt increase in loading to the foot upon collision with the ground – not observed in forefoot-striking runners. These effects are shown below in Figure 1. Although Lieberman et al. calculated the effective mass colliding with the ground for heel and forefoot striking, they did not make direct measurements of loading distribution on the plantar surface for these different landing styles (2010).

Modern running shoes tend to feature more heel cushioning and arch support. Heel cushioning distributes the impact force over a larger area while also absorbing some of the impact upon collision; meanwhile, arch support reduces the flattening of and loading to the arch of the foot. Shoe cushioning is often identified as an explanation for the high rate of heel striking among shod runners because this cushioning makes heel striking more comfortable, as evidenced by the finding that heel cushioning decreases the impact transient by 10% as well as reducing the loading rate. Cushioned running shoes also tend to have thicker heel cushioning than forefoot cushioning, giving them “heel-toe lift” or “heel-toe drop” which further increases the likelihood of the heel striking first (Lieberman et al., Biomechanics of Foot Strikes & Applications to Running Barefoot or in Minimal Footwear).

The minimalist running movement suggests running barefoot or running in less supportive shoes in order to encourage a forefoot strike. Some less supportive shoes simply have less cushioning; others are designed to keep the heel and forefoot at the same level by using the same thickness of cushioning across the foot. This latter minimalist shoe feature is termed “zero drop” because the typical running shoe’s heel-toe drop is absent.

Besides switching to a forefoot strike as advocated by the minimalist movement, another common method of trying to prevent injury from loads imparted while running is choosing softer running surfaces; however, a previous study involving running trials on asphalt, concrete, grass, and a synthetic track found no significant differences in shoe reaction forces, contact time, or impulse among the different surfaces. The authors of this study suggested internal compensatory mechanisms may instead be responsible for increased injury risk on harder surfaces, but the experiment did not include an investigation of load distribution on the plantar surface nor did it study heel versus forefoot striking on the surfaces tested (Tillman et al., 2002). Another study, conversely, found statistically significant differences in load distribution across the plantar surface between trials conducted on asphalt and those conducted on grass. Again, these patterns were not tested for heel versus forefoot striking (Tessutti et al., 2010).

A final loading consideration in running and subsequent injury risk is velocity. Increasing ground reaction forces have been shown to correlate with increased running velocity. These results were obtained from a study of heel striking runners (Hamill et al., 1983). The following experiment addresses the effects of velocity change on shoe reaction forces in both heel and forefoot striking.

**In-Shoe Measurement of Load Imparted to Plantar Surface**

The load imparted to the lower surface of the subject’s foot was measured using force sensors attached to the ball, arch, and heel of the foot. Running trials of 7

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**Figure 1.** Heel striking produces a large impact transient in the ground reaction force. Forefoot striking eliminates this impact transient. Results were obtained from trials of barefoot running (Lieberman et al., 2010).
seconds each were conducted under different shoe type, velocity, and terrain conditions. One foot strike from each trial was recorded using a video camera for visual analysis of the landing style. Average impact loads to each area of the foot were calculated for each condition.

**Force Pads & Calibration**

Interlink 406 (IL406) force sensing resistors (Figure 2) were used to measure the load distribution to the plantar surface. IL406 force pads consist of variable resistors that decrease in resistance as force increases. The force pads were each incorporated into a voltage divider circuit supplied by a 9V battery, where the output voltage across the fixed resistor in each circuit is related to the force on the sensor by a calibration curve as shown in Figure 3.

Calibration curves for each IL406 force pad were obtained by placing the IL406 on top of a Vernier Force Plate, which measures force, hooking both the force plate and the force pad up to a Vernier Lab Pro Data Acquisition (DAQ) Interface, and pressing on the IL406 force pad such that both the force pad and plate experienced the same force. A curve was fit in Logger Pro to the force measurements from the Vernier Force Plate as a function of the voltage measurements from IL406. The fit equations were used to convert voltage measurements for each IL406 sensor to force measurements prior to data analysis (Hughey and Hunter, 2011).

**In-Shoe Force Sensor Setup**

The subject was a healthy 19-year-old female runner who ran with a heel strike until switching to a forefoot strike two years prior to participating in this study. IL406 force pads were gently taped using athletic tape to three locations on the bottom surface of the subject’s left foot: the ball (medial forefoot), arch, and heel (central rearfoot). More tape was placed over each of the sensors and their wire connections to prevent tearing as the subject took running shoes on and off. The wires from the force pads were taped along the subject’s leg to prevent interference with the running motion, and each sensor was connected to a Vernier Lab Quest 2 wireless DAQ interface. The experimental setup is shown in Figure 4.

**Running Trial Procedure**

Running trials were conducted using three types of running shoes: New Balance 759 (Women’s size 7.5, neutral cushion), Nike Free Run+ 2 (#443816-806, Women’s size 7), and Altra Torin (#A2235, Women’s Size 7). The New Balance model is a more standard modern running shoe with arch support and heel cushioning, whereas the Nike Free is a minimalist shoe that is more flexible and lightweight. Another minimalist running shoe, the Altra, eliminates the arch support and heel-toe drop (the difference in cushioning thickness that causes the heel to be higher than the forefoot) found in the Nike Free and New Balance models.
The subject wore the Altra running shoes for trials comparing terrain and velocity effects. Three running surfaces were tested: a stretch of smooth asphalt pavement along a neighborhood street, grass in a field, and gravelly dirt located between a park and a paved roadway. Velocity conditions included four of the subject’s self-selected speeds: slower than regular training pace, regular training pace, fast training pace, and full-out sprint. For all other trials, the subject was instructed to run at her regular training pace. Trials comparing shoe and velocity effects were conducted on the asphalt surface.

All conditions were tested in separate trials for forefoot and heel striking. In each running trial, the subject was instructed to run for approximately seven seconds, and data were sampled using the Vernier Lab Quest 2 at a rate of 1000 Hz for a duration of seven seconds. One left foot strike from each trial was videotaped using a SONY HDR-SR8 video camera.

Data analysis

Data from the first two seconds and final second of each trial were discarded due to variations in stride as the subject accelerated to steady-state gait at the beginning of the trial and decelerated to a stop at the end of the trial, respectively. The subject was typically able to complete five to eight foot strikes within the analyzed time frame. Within each trial, the values for the peak impact force from the five to eight individual foot strikes were averaged to obtain a single mean value for each the ball, arch, and heel of the foot.

Visual inspection of the video recordings was performed to confirm the landing style employed in each trial. Reported uncertainty values represent the precision uncertainty for the mean values at the 95% confidence level using the t-statistic approach. Paired, two-tailed Student’s t-tests were performed using the first six foot strikes of each trial to obtain p-values.

Results and Discussion

In this experiment, we observed the large impact transient previously observed in heel striking (Lieberman et al., 2010). Compared to forefoot striking, heel striking produces significantly higher peak impact forces to the arch and heel. Results also show a significant reduction in loading to the plantar surface in more cushioned running shoes as well as on softer terrain. Contrary to previous findings (Hamill et al., 1983), we observed a decrease in location-specific peak impact forces with increasing velocity.

Representative images of the subject’s shod foot upon striking the ground are given in Figure 5 for forefoot and heel striking. As seen below, in forefoot striking, the subject’s forefoot lands first, to be followed by the heel (this stage not pictured), whereas in heel striking the subject’s rearfoot clearly lands first.

Figure 5. Collision of the subject’s foot with the ground in forefoot striking (left) and heel striking (right). Images were taken from video capture during trials conducted on asphalt in Altra running shoes.

Figure 6. Peak load distribution on the plantar surface in forefoot (blue) and heel (yellow) striking. Trials were conducted on asphalt in Altra running shoes at the subject’s regular training pace. These data represent an average of individual foot strikes from six independent trials that were conducted for each forefoot and heel striking under these conditions.
As shown in Figure 6, our results indicate that heel striking yields much higher loading to the plantar surface than forefoot striking. The impact force imparted to the forefoot is slightly higher in forefoot striking, as was expected since the forefoot is the first to collide with the ground in this landing style, whereas in heel striking the forefoot lands after the heel has made the initial collision. On the other hand, the impact forces transmitted to the arch and heel are much higher in heel striking. The loading to the heel of the foot is 2.8 times higher in a heel-toe running style than in a toe-heel-toe landing style.

The source of the increased loading in heel striking is likely due to the large impact transient which has been observed previously (Lieberman et al., 2010) and which was also observed in this experiment as seen below in Figure 7.

The large impact transient – displayed in red for the loading to the heel – present in the graph on the right of Figure 7 is due to the collision of the heel with the ground at the beginning of a heel-toe landing. This impact transient is absent from the graph of toe-heel-toe landing style data on the left. Total load, calculated by summing the force imparted to the forefoot, arch, and heel at each time point, is shown in Figure 8.

The total loading to the foot measured in this experiment is reduced, on average, by nearly 29% in forefoot striking as compared to heel striking. Although the peak loads observed in this experiment were lower than those found in previous studies, (Tillman et al., 2002) this observation may be attributed to the fact that this experiment analyzed loads imparted to specific areas on the plantar surface – only accounting for less than two-thirds of the total area – rather than the plantar surface as a whole. Furthermore, as opposed to having the subject run on a force plate, this study used in-shoe sensors, which allow the measured impact force to be reduced by the damping and distributing effects of the running shoe.

One limitation encountered in this experiment was the susceptibility of each trial to inconsistencies introduced by the subject in her running style and speed. Since the data in Figure 6 represent mean values obtained from foot strikes in six independent trials, the uncertainty values displayed in the error bars account for variations among individual trials. The uncertainty values are given in Table 1.

The loading to the forefoot and arch, as shown by the small uncertainty values for these locations, is relatively unaffected by variations in the subject’s running style from trial to trial. Heel loading, particularly in the case of forefoot striking, exhibits less consistency among trials as indicated by the 12% uncertainty in the mean value of the peak load. This inconsistency is still relatively low, but video analysis yields a possible explanation for the value. During forefoot trials, the subject’s landing style occasionally exhibits midfoot strike characteristics.
Since midfoot striking involves simultaneous landing of the heel and forefoot, collisions of this nature produce higher loads to the heel than those observed in forefoot striking. Occasional midfoot strikes would introduce higher variation in peak loading, particularly to the heel of the foot (Lieberman et al., Biomechanics of Foot Strikes & Applications to Running Barefoot or in Minimal Footwear).

Comparison of results from trials in the three shoe types reveals a clear reduction in loading across the plantar surface in the New Balance and Nike Free shoes as seen in Figure 9. For the New Balance and Nike Free shoes, the forces transmitted to the arch in forefoot striking and to the heel in both forefoot and heel striking are within the same 95% confidence level ranges.

Table 1. Uncertainty values for the mean peak force imparted to three locations on the plantar surface. The mean values were calculated from individual foot strikes from six independent trials conducted on asphalt in Altra running shoes at regular speed.

Figure 9. Peak load distribution to the plantar surface for forefoot striking (left) and heel striking (right) under different running shoe conditions. Data from the Altra (blue), New Balance (red), and Nike Free (yellow) shoes are shown. Trials were conducted on asphalt at the subject’s regular training pace.

Table 1. Uncertainty values for the mean peak force imparted to three locations on the plantar surface. The mean values were calculated from individual foot strikes from six independent trials conducted on asphalt in Altra running shoes at regular speed.

Figure 10. Peak load distribution to the plantar surface for forefoot striking (left) and heel striking (right) under different terrain conditions. Trials were conducted in Altra running shoes on asphalt (gray), grass (green), and dirt (yellow) at regular training pace. The asterisk (*) represents a p-value less than 0.05.
The similar level of cushioning and arch support in the New Balance and Nike Free models likely accounts for the similarity in arch and heel loading between these two shoes. The absence of arch support and extra heel cushioning explains the significantly higher arch and heel loading observed in the Altra. The difference in forefoot loading, though significant across all conditions, is much smaller than that observed in other locations on the plantar surface. This result may occur because cushioning thickness under the forefoot is not increased as much as that under the heel in the New Balance and Nike Free shoes, giving these models the characteristic heel-toe drop which is absent in the Altras. Averaging the more supportive New Balance and Nike Free models together and comparing them to the Altras, increased shoe support led to total impact force reductions of nearly 26% and 36% for heel and forefoot striking, respectively.

The effects of terrain on shoe reaction force are presented in Figure 10. Loading to the forefoot and heel for both landing styles, as well as loading to the arch in heel striking, is significantly higher on asphalt than on dirt or grass. Running on dirt or grass instead of asphalt decreases total loading by 15% for heel striking and 16% for forefoot striking. Results for trials on grass are not significantly different from those on dirt except in the case of load imparted to the heel during toe-heel-toe running. On the other hand, loading across the plantar surface is significantly reduced on grass versus asphalt for both forefoot and heel striking, the only exception being loading to the arch during heel striking.

These results for asphalt versus grass are consistent with those obtained by Tessutti et al. (2010) for the forefoot and rearfoot regions, although that study investigated the lateral forefoot, whereas this study focused on the medial forefoot. It should be noted that Tessutti et al. did not report results specific to forefoot and heel striking subjects (2010).

Contrary to expectations of increasing loading with increasing velocity (Hamill et al., 1983), the shoe reaction force during the full-out sprint was not significantly higher than that during a slower speed trial for any location on the plantar surface in either forefoot or heel striking as shown in Figure 11.

Instead, the loads imparted to all three locations on the plantar surface during the sprint trials were

Figure 11. Peak load distribution to the plantar surface for forefoot striking (left) and heel striking (right) under different velocity conditions. Trials were conducted in Altra running shoes on asphalt at four of the subject’s self-selected speeds: slower than regular training pace (purple), regular training pace (yellow), fast training pace (red), and full-out sprint (green). The asterisk (*) represents a p-value less than 0.05.

Figure 12. Collision of the subject’s foot with the ground at sprint speed (left) and at slow speed (right). Images were taken from video capture during forefoot striking trials conducted on asphalt in Altra running shoes.
significantly lower than those during the regular-speed trials. Moreover, the arch and heel loading were significantly lower during the fast forefoot striking trial than during the corresponding regular speed trial. One possible explanation for these unexpected results in velocity effects is the midfoot striking occasionally observed in forefoot striking trials. As speed increases, runners tend to run farther forward on their feet, decreasing the likelihood of the occasional midfoot striking. The intermittent midfoot striking at lower speeds observed in video capture from these trials, as shown in Figure 12, may account for the higher loads observed across the plantar surface at lower speeds.

Characteristic of midfoot striking, the forefoot and heel land nearly simultaneously in the image taken from a slow speed trial (right of Figure 12). The heel is significantly higher off the ground during the sprinting strike (left of Figure 12) than during the slow strike. As for the results observed in heel striking, it is possible that the high speed of the sprint causes more of the leg’s vertical momentum to be converted to rotational momentum, thus reducing the impact peaks.

Aside from variations in the subject’s running stride and velocity from trial to trial, the other primary limitation of this experiment is the effect of force pad sensor location on the results obtained. For instance, if the IL406 force sensors are placed more laterally or medially on the ball, arch, or heel of the foot, the loading results may change significantly. The effects of changing sensor location are presented in Figure 13.

![Figure 13. Peak load distribution to the plantar surface for two different sets of sensor locations: one more medial (blue), the other more lateral (yellow). Trials were conducted in Altra running shoes on asphalt at the subject’s regular training speed.](image)

The more lateral IL406 placement yields significantly lower loading across the plantar surface than placement a few millimeters more medial; however, although the heel loading in the medial location is about 2.8 times higher than that in the lateral location, the increase in loading for the medial location is similar for the forefoot (1.3 times that for the lateral location) and arch (1.4 times that for the lateral location).

Conclusions

Results of this experiment confirm the large impact transient observed previously in heel-toe style running (Lieberman et al., 2010). This confirmation, in combination with the higher loading to the arch and heel observed during heel striking, supports the idea that running with a forefoot strike reduces injury risk by decreasing load imparted to the plantar surface.

The strategy of preventing injury by running in more cushioned and supportive shoes is confirmed by shoe reaction forces, as shown by findings that loading is significantly lower in the New Balance and Nike Free shoes as compared to the Altras; however, other factors such as the effects of running in supportive shoes on foot strength and susceptibility to injury must also be considered in the running shoe debate. The effects of terrain on loading are significant as well; namely, running on softer surfaces like grass or dirt significantly decreases peak impact loads, thus potentially also reducing injury risk, in both toe-heel-toe and heel-toe running.

Further investigation is recommended to better clarify the relationship between peak impact loads and injury risk. In addition, further studies of the relationship between running shoe cushioning and support and injury susceptibility are necessary in order to draw conclusions about the safest choice of shoes. Although varying running speed is correlated with a much smaller trend in decreasing impact loads, our results indicate that loading – and, thus, potentially injury risk – can be reduced more effectively by selecting a softer running surface, selecting appropriate running shoes, and adopting a forefoot strike.

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References


Kicking off a tradition
By Ebaa Al-Obeidi, MURJ Co-Editor-In-Chief

This year, MURJ collaborated with MIT’s chapter of Sigma Xi to spearhead MIT’s first interdisciplinary undergraduate poster session. Our goal was to give undergraduates the valuable experience of communicating their research to a diverse audience. We were also eager to showcase to prospective students the tremendous opportunities for research that MIT offers.

I am proud to report that we succeeded on both fronts. We had a diverse group of 21 presenters who drew from all four grade levels and represented eight departments from across the Schools of Science and Engineering. Judges from MIT’s Postdoctoral Association also came from several different research backgrounds and were impressed by the caliber of the research. Over 100 people attended and many prospective students left the event excited and inspired.

MURJ and Sigma Xi look forward to making this event an annual tradition, and we encourage everyone reading this to participate next year. Learning to communicate research is a critical part of our education at MIT, and this event provides a platform to hone this crucial skill.

Finally, I’d like to acknowledge the fantastic MURJ/Sigma Xi student team that made this event possible, the judges from the MIT Postdoctoral Association who kindly gave their time to give invaluable feedback to the students, and the presenters for their enthusiasm and excitement. Congratulations to our first place winner Pratheek Nagaraj ’16, our second place winner Sarah Guthrie ’14, and our third place winners Khristian Bauer-Rowe ’14, Andrea Kriz ’15, and Debra Van Egeren ’16.